Developing a Practical Methodology for Chinese Medicine Research
- Approach, Challenges and Solutions

by

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Abstract

Traditional Chinese Medicine (TCM) enjoys a long history dating back to the era of *Huangdi* (黃帝)(2698-2598 BC). Up to now, TCM has existed more than 4700 years. The development of TCM has experienced four brilliant and prosperous periods. The *Han dynasty* (206BC—AD 200) is considered the most glorious epoch in Chinese medicinal history. *Zhang Zhongjing* (張仲景) and *Hua Tuo* (華佗) were the outstanding representatives in this period. In the *Jin-Yuan dynasty* (金元時期), TCM theories were remarkably improved. During this period, there were four outstanding Chinese medicinal practitioners: *Liu Wansu* (劉完素), established the "cooling school"; *Zhang Zhihe* (張子和), created the "attacking school"; *Li Dongyuan* (李東垣), established his famous thesis that most diseases were due to injury to the stomach/spleen system; and *Zhu Danxi* (朱丹溪), who was good at temperance and use of tonic formula. In the *Ming dynasty* (AD 1368-1644), TCM further improved. The most outstanding contribution was the publication of *Ben Cao Gang Mu* (本草綱目) by *Li Shizhen* (李時珍). In the *Qing dynasty* (AD 1644-1911), the *Wen Bing school* was founded. The most important contributors were *Wu Youke* (吳又可) (瘟疫論), *Ye Tianshi* (葉天士) (溫熱論), and *Wu Jutong* (吳鞠通) (溫病條辨).

During the time-honored history of several thousand years, numerous valuable experiences and literatures were recorded. According to current EBM requirements, classical records alone are insufficient to proof the efficacy of TCM. A strong evidence of effectiveness should come from a well-designed clinical trial that follows the randomized, double-blinded, placebo-controlled clinical trial principles. The purpose of the research is to develop a practical methodology to obtain convincing evidences in quality, safety and efficacy of Chinese Medicine.

When we use evidence-based research methodology to prove the quality, safety and efficacy, we are facing many challenges.

The distinctiveness of Chinese medicine is manifested in the diversity and the

complexity of its components, the instability of its quantity, the fuzziness of its action mechanism, and the uncontrollability of its producing process. Traditional Chinese herbal formulae are usually formed by more than one plants, animal or mineral items. The composition is extremely complex. The efficacy thus can hardly be guaranteed. The methods of harvesting, drying, storage, transportation, and processing of plant material influence the efficacy and safety. The consistent efficacy, therefore, can hardly be guaranteed. For thousands of years, it has been observed by clinical practice that Traditional Chinese Medicine (TCM) has a rich scientific connotation and has developed a unique healthcare system. However, variable sources of raw materials, unknown active ingredients, difficulties in quality control, lack of safety evaluation, unclear mechanism of action, etc., all these factors constitute major challenges in modernization of TCM.

The general opinion believes that traditional Chinese medicine is developed from nature with thousands of years of human application experience, so their safety should be guaranteed. The general perception that herbal drugs are very safe and free from side effects is not true. Little do they know in the modern conditions, that the current herbs used for preparation of traditional Chinese medicine have been very different as compared with the ancient herbs in planting, extracting, producing, storage, application, dose level and the duration of clinical use The active ingredients of herbal formula are higher after extracting with modern scientific methods; and the toxicity may also be correspondingly higher. The potential side effects after long-term use should not be overlooked.

Research of traditional Chinese medicine is a complex subject, and many factors affect the research process and results. For example, the complex chemical composition of Chinese herbal medicine is the material basis for multi-target therapy, but the complex chemical composition can cause great difficulties in the active ingredient identification and quality control. The quality control of Chinese herbal medicine is a systematic procedure. The initial critical step is to standardize the starting raw herbs, for knowing the exact species and subspecies, the ideal growing location, environmental conditions, harvesting methods, and storage conditions etc.,

i.e., following Good Agriculture Practice (GAP) to ensure the quality of the raw materials. The second step is to standardize the processing methods of the raw herbs. The third step is to standardize the preparation procedure of the final products according to the requirements of Good Manufacture Practice (GMP) guideline. The fourth step is to qualitatively and/or quantitatively evaluate the quality of the TCM medication based on one or more selected chemical markers.

The effect of Traditional Chinese Medicine (TCM) may be characterized by its chemical compounds, which are also the active ingredients. The reproducibility and the stability of the active ingredients are the foundation to ensure the efficacy of TCM. The safety and efficacy of TCM is evaluated through its pharmacological effects and in clinical studies. Many Chinese herbal medicines have a long history of traditional use. However, most of them are of unproven efficacy by today's standard. Well-designed randomized controlled trials and comprehensive pre-clinical studies are not known. Although the lack of qualified evidence does not mean that Chinese herbal medicines lack efficacy or are unsafe, properly designed experimental and clinical investigations should still be done today. There is a need to scientifically prove and clinically validate its safety and efficacy through chemical standardization, biological assays, and clinical trials.

Chemical analyses, biological assays, and animal experiments provide important fundamental information of Traditional Chinese Medicine. In modern biomedical research, they should also be necessary prerequisites for clinical trials.

A Chinese herbal formula, no matter how effective and safe in the pre-clinical studies, cannot be assumed clinically valid. No marketing approval should be granted without clinical studies. Thus, clinical trial plays a decisive role in the research and development of Traditional Chinese Medicine. Currently the highest level of efficacy evidence is obtained from randomized controlled clinical trial. Thousands of years of traditional use can provide us with valuable guidelines to the selection, preparation and application of herbal formulations. To be accepted as viable alternatives to

western medicine, the rigorous methods of scientific and clinical validations must be applied. Our experiences have demonstrated that it is feasible to perform well-designed TCM clinical trials, although there are challenges to face, for example placebo preparation, outcome measurement and batch-to-batch consistency of TCM medication.

In conclusion, the methodology of Chinese medicine research needs to improve. One practical way is to apply the efficacy-driven approach through the following steps: i) getting a simple herbal formula to try solving a difficult clinical problem and start an evidence-based clinical trial using methodology acceptable to current standard clinical trials i.e. randomized, placebo-controlled; ii) parallel laboratory experiments to understand the mode of action should be done; and; iii) making sure that the quality of herbs and their extracts are of the best standard. A promising item shown in the clinical trial and laboratory experiments would deserve more pharmacological investigations before considering marketing.

摘要

中醫藥歷史悠久，可以上溯到*黃帝*時期(2698-2598BC)，迄今已有 4700 年。歷史上中醫藥的發展經歷了四個輝煌時期。第一個輝煌時期在漢朝(206BC—AD200)，*張仲景*和*華佗*是這一時期的傑出代表。第二個輝煌在金元時期，中醫藥理論在這一時期得到顯著發展，產生了著名的金元四大家----寒涼派的*劉完素*、攻下派的*張子和*、補土派的*李東垣*和滋陰派的*朱丹溪*。第三個輝煌在明朝，這一時期中醫藥得到進一步發展。最突出的貢獻是*李時珍*的《本草綱目》的誕生。第四個輝煌時期在清朝，最明顯的標誌是瘟病學說的創立，包括*吳又可*的《瘟疫論》、*葉天士*的《溫熱論》及*吳鞠通*的《瘟病條辨》。

數千年的發展過程中，中醫藥積累了豐富的經驗，留下了大量珍貴的文獻記錄。然而，從現代循証醫學的角度看，以傳統經驗的累積及個別案例的記錄所得的證據，不足以形成令人信服的依據。目前認爲，最有說服力的療效證據來自設計合理的隨機、雙盲、安慰劑對照的臨床研究。本研究的目的是探求可行的研究方法，獲得中藥在品質、安全性及有效性方面的可靠證據。

以目前對療效證據的要求標準來看，中醫藥研究應該引入循證醫學的研究方法，才能獲得主流醫學認可的療效證據。然而，在研究過程中，我們會面對特殊的困難和挑戰。

中藥最明顯的特點是其多樣性和複雜性，表現爲組成成分複雜、品質不穩定、作

用機理不明確、生產製作過程難控制等。中藥複方通常由多種中藥材組成，有些複方還含有動物和礦物成份，因此組成成份極其複雜。此外，藥材採收、乾燥、儲存、運輸及加工製作過程，以及提取方法，使用的溶劑，有效成份的穩定性等因素，均會影響療效和安全性。以上種種因素增加品質控制的難度，使中藥療效穩定十分困難。

儘管中醫藥來自臨床實踐，代代相傳，但是以目前循証醫學的觀點看尚缺具有說服力的證據。隨著時代的變遷，祖先留下的豐富中醫藥遺產是否也需要按當代科學發展的要求和標準，進行重新評價，是需要認真考慮的問題。

普遍的觀點認爲，中藥來自天然且已使用數千年，安全性應該有保障。不難設想目前使用的中草藥與古代的中草藥在種植、提取、生產、儲存、臨床使用、用量及使用時間等方面已經存在巨大的差別。經過現代科學方法提取後，中藥的有效成份更高了，毒性成份也相應增加。長期使用引發的潛在副作用不能忽視。安全性的再評價變得更有必要。

很多中藥已使用很長時間，但是大多數尚未經過設計完善的隨機對照臨床試驗來證實其療效。僅靠應用歷史悠久尚不足以成爲安全性的評價標準。不過，缺乏現代醫學標準下的安全性和有效性證據，並不意味中藥無效或不安全，但嚴格設計的試驗研究和臨床試驗依然需要完成，同時應探討適合中藥特點研究方法。迄今爲止，研究中醫藥的方法仍然是借鑒對西藥研究的方法。這是否是研究中藥的最佳方法，值得探討。

許多因素可以影響中藥研究進程和研究結果。其中一些因素對中藥來說是優勢，從另一角度看，這些優勢又成爲研究中藥的障礙。如中藥複雜的化學成份是多靶

點治療的物質基礎，但對活性成份的鑒別和產品質量控制又帶來很大困難。中藥質量體現在五個範疇：藥材質量控制(GAP) 、安全性評價(GLP) 、生產過程質量控制(GMP) 、標準化生產規範，及藥理學活性劑量。對這些環節的質量控制首要及關鍵性步驟是藥材的標準化，如確切的種屬、生長地域、生長環境、採收方法及儲存條件等，對這些環節的控制，才能確保原藥材的質量穩定；其次是藥材加工程式的標準化；第三是藥品成品生產過程的標準化(GMP) ；最後是根據選擇的化學標準品對中藥質量進行定量或/和定性測定。

中藥的療效可能由其化學成份所決定。決定中藥療效的化學成份稱爲活性成份。活性成份的可重複性和穩定性是保障中藥療效及穩定的基礎。中藥的安全性和療效的評價基於藥理學作用和臨床研究的結果。許多中藥有相當長時間的人體應用傳統，然而，其中的大多數依然缺乏現代意義上的療效評估，即設計嚴謹的隨機對照試驗和全面的臨床前研究。雖然缺乏目前公認的證據，這並不意味中醫藥無效或不安全。但是有必要進行設計嚴謹的科學試驗及臨床驗證，從化學成份、生物學試驗、動物模型及臨床試驗等方面證實中藥的安全性和療效，提高中藥安全有效證據的可靠性。

一個中藥複方，無論臨床前的藥理學研究顯示如何有效，也無論動物試驗結果證明如何安全，如果沒有臨床試驗數據的支持，其安全性和有效性便不會被最終認可，也不可能獲得上市許可。因此，臨床試驗在中醫藥的研發中扮演著決定性作用。目前認可的最高水準的有效證據來自隨機對照的臨床試驗。數千年的中醫藥臨床實踐給我們留下珍貴的遺產，爲我們選擇、製備和應用中藥提供了重要參

考。爲了符合主流醫學的標準，有必要進行嚴格設計的科學研究和臨床驗證。我們以往的研究經驗表明，進行設計嚴謹的中藥臨床研究是可行的，雖然其中會面臨許多的挑戰，例如安慰劑的製備、結局指標的選擇、不同批次中藥質量的穩定性等等。

總而言之，中醫藥研究的方法需要改進，切實可行的方法是以療效帶動研究。首先根據文獻記載和現代研究結果，組成儘量簡單的中藥複方，針對臨床上的難題，以循証醫學研究爲原則，以隨機對照臨床試驗爲手段，證實該複方的安全性和療效。當療效得到證實後即進行實驗室研究，探討產生療效的機製和作用模式，同時對中藥質量及提取方法進行研究，制定質量標準。最後，綜合臨床前和臨床研究資料，準備註冊檔申報上市。

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ABBREVIATIONS

AD	Atopic Dermatitis
ADL	activities of daily living
ADMET	Administration, distribution, metabolism, excretion, and toxicological studies
ADP	Adenosine Diphosphate
AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
ANOVA	Analysis of Variance
AR	Allergic Rhinitis
AST	Aspartate aminotransferase
BDFI	Bioactivity-directed fractionation and isolation
BMD	Bone Mineral Density
BPH	Benign Prostatic Hyperplasia
CDLQI	Children's Dermatology Life Quality Index
CHM	Chinese herbal medicine
CM	Chinese Medicine
CMC	Chemistry-manufacturing-control
CMM	Chinese Materia Medica
CONSORT	Consolidated Standards Of Reporting Trials
CRF	Case Report Form
CS	Corticosteroids
DBT	Danggui Buxue Tang
DDT	Dichlorodiphenyltrichloroethane
DSHEA	Dietary Supplement Health and Education Act
D&G	Danshen and Gegen
DMF	Drug Master File
DNA	Deoxyribonucleic acid

EBM	Evidence-based medicine
EC	European Communities
ELP	Epimedii, Ligustri Lucidi, Psoralea
FDA	Food and Drug Administration
FMD	flow-mediated dilation
FSH	Follicle stimulating hormone
GAP	Good Agricultural Practice
GC-MS	gas chromatography- mass spectrometry
GCP	Good Clinical Practice
GEP	Good Extracting Practice
GHQ	General Health Questionnaire
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GTN	Glyceryl trinitrate
Hb	Haemoglobin
HC	H. corymbosa (L.) Lam.
HD	Hedyotis diffusa
HER	herb to extract ratio
HIV	Human immunodeficiency virus
HKIB	Hong Kong Institute of Biotechnology
HPLC	High performance Liquid Chromatography
HPCE	high performance capillary electrophoresis
HRT	Hormone Replacement Therapy
HSCCC	high speed countercurrentchromatography
IACP	Image Analysis Computer Program
IBS	irritable bowel syndrome
ICM	Institute of Chinese Medicine
IMT	intima-media thickness
IND	Investigational New Drug

IP	intellectual property
IPSS	International Prostate Symptom Score
ITT	Intention-to-treat
LD50	median lethal dose
LDH	lactate dehydrogenase
LH	luteinizing hormone
MCID	minimally clinically important difference
MCV	Mean corpuscular volume
MENQOL	Menopause-Specific Quality of Life
MO	Mononuclear leucocyte
MND	motor neuron diseases
MPV	Mean Platelet volume
MTT	methyltetrazolium
NCCAM	National Center for Complementary and Alternative Medicine
NDA	prescription drug, New Drug Application
NICPBP	National Institute for the Control of Pharmaceutical & Biological Products
NOAEL	No observed adverse effect level
OAM	Office of Alternative Medicine
pCm	proprietary Chinese medicines
PHA	Phytohaemagglutinin
PRO	patient reported outcomes
PSA	prostate specific antigen
OTC	Over The Counter
OVX	ovariectomized
QC	Quality Control
QoL	Quality of Life
RA	Radix Astragali

RAS	Radix Angelicae Sinensis
RBC	Red blood cell
RCCT	Randomized controlled clinical trial
RCT	Randomized Controlled Trial
R & D	Research & Development
SAP	Statistical analysis plan
SATCM	State Administration of Traditional Chinese Medicine
SBL	Shi Bi Ling
SCORAD	SCORing of Atopic Dermatitis
SD	Sprague-Dawley rat
SFC	supercritical fluid chromatography
SFDA	State Food and Drug Administration
SF-36	Short Form (36) Health Survey
SPSS	Statistical Package for the Social Sciences
STZ	streptozotocin
TCM	Traditional Chinese Medicine
TCHM	Traditional Chinese Herbal Medicine
CHOL	Total Cholesterol
TG	Triglycerides
TLC	Thin Layer Chromatography
TMP	Trimethoprin
TP	Total Protein
VAS	visual analogue scale
WBC	White blood cell
WHO	World Health Organization
WM	Western Medicine

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Chapter 1

Introduction

Traditional Chinese medicine has a long history; it has played a great role in the generation and boom of Chinese people. In the long course of history, after numerous attempts and failures, Traditional Chinese medicine gradually developed an integrated and theoretical system, and effective therapeutic methods. Reviewing the course of the development of Traditional Chinese medicine, we can stand on the level of history, to objectively understand the role of Traditional Chinese medicine in healthcare and to objectively evaluate its effectiveness.

1.1 History of Traditional Chinese Medicine

The Origin of Traditional Chinese Medicine

According to the investigations of anthropologists, one species of direct ancestors of human beings was *Lamagu* ape which lived about 14 million years ago (1, 2). Their fossil was discovered in *Kaiyuan* (開遠縣) and *Lufeng* (祿豐縣) counties in *Yunnan* Province, which means that China is also an original place where human beings developed.(1) A large number of objects underearth were found. Most of them were medicine related materials, from which the living environments at that time could be deduced (3). From these unearthed objects we can imagine that our ancestors were facing very serious natural environments that seriously influenced their health. The average age of people at that time was extremely short. The harsh natural environments forced them to explore every possible means to relieve and prevent

diseases. "In ancient time, birds and beasts were everywhere but human beings were very few. So people had to live on trees to avoid dangers or threats. They collected oak millet for alleviating hunger in day time, and slept on the trees at night." recorded by *Zhuangzi* 莊子 (369 ～ 286 BC) in his chapter named *Dao Tuo Bian* (盜拓篇).

Fire is one of the natural phenomena, but it took a very long time that human beings became to recognize, control and make use of it. Apart from cooking, one of the most important characteristics of fire in relieving diseases was found by our ancestors. The discovery and utilization of fire not only enabled people to consume the cooked food, but also shortened the time of digestion, that made people easily absorb more nutrient substances that were good for the development of human body and brain. Through countless practices people also noticed that using hot soil or sand, wrapped by hide or bark to warm abdomen or joints, could relieve pain. This might be the beginning of thermotherapy. Although there is no complete evidence of its history of development in the prehistorical and extremely primitive days, it is believed that it must be closely related to the eating habits of ancient people and their observations of animal behaviour, based on that the primitive medicine originated (4).

Usually it is believed that the traditional acupuncture therapies come from a *Stone needle* (砭石). According to the archaeological findings at *Duo Lun County* (多倫旗) in *Inner Mongolia* (內蒙古) in 1963, *Stone needle* was originated in *Stone Age*, about 14 million years ago. *Stone needle* was the instrument that ancient people used to fight with diseases. It was used for pain relief or bleeding or in discharging pus (5).

In 1968, *Nine-Kinds Needles* (九針) were discovered from the *Western Han Dynasty Tomb* in *Mangcheng* (滿城) of *Hebei Province* (河北省). The *Yellow Emperor* (黃帝內經) described that needle was developed into nine kinds; each kind has its own particular use. The technique of puncture had been a remarkable innovation in China. However, we are not able to know exactly when the Chinese started to use herbal medicine (5,

6).

Historical records of Chinese herbal medicine started with references from *Shen Nong* who lived about 5000 years ago (7, 8). After thousands of years in the use of herbal medicine and the accumulations of numerous experiences in therapeutic practice, the first book of Chinese herbal medicine, *Shennong Bencao Jing* (神農本草經) was completed. The book recorded 365 types of medicine; some of them are still used nowadays in clinics and hospitals. From then, a rigorous, systematic subject of Chinese medicine began to take place (9, 10). China government has announced an ambitious plan to modernize the ancient practice of traditional Chinese medicine (TCM). It will push the basic and clinical research, and improve the testing and developing of TCM globally. But the critical question is whether the research could meet the scientific standards necessary for international recognition (11).

It is impossible to tell the exact time when Chinese medicine started taking shape, but ancient legends seem to trace its beginning to the *Stone Age* when Chinese transformed from hunters to farmers (12). From that time, they had accumulated sufficient experiences to use nature to their advantage. *Shen Nong's* story of "tasting herbs" (神農嚐百草), for example, reflected how "man gradually learned to recognize the properties of plants" after experiencing innumerable failures (13,14). Most of scholars believe that Chinese Medicine dated back to the era of *Huangdi*, the *Yellow Emperor* (2698-2598 B.C). Although scattered records of herbal knowledge can be found in early works such as *Zhou Li* (周禮, c 4th century BC) and *Er Ya* (爾雅 426-221 B.C), it was not until the *Eastern Han dynasty* (東漢) of China (25-220 AD) that the first book systematically describing herbs, *Shennong Bencao Jing* (神農本草經) is believed to have appeared (15). The responsibilities of medical doctors were defined in the early *Zhou dynasty* (1122-221B.C.), which was a great achievement in intelligence. The *Zhou dynasty* was one of the most outstanding periods in Chinese

history. Literature, art, religion, philosophy, and all that are usually included in the term civilization in this phase, flourished and reached a high degree of development. Some very important discoveries of Chinese medicine were made during the *Zhou dynasty*, including the theoretical foundations of *yin* and *yang*, the *five elements*, the pathogenic factors of external environment as causes of disease and further understanding of the meridians of acupuncture (16). Basically medicine was an applied, practical and effective art that was based on observations and experiences, and it adopted the theories of *Yin* and *Yang*, and the *wu-xing* (five elements, 五行) concept. It was believed that health was a status in which the body maintained a relative balance, not only internal but also with the external environment (17). Diagnosis (such as inspection 望, listening and smelling examination 聞, inquiring 問, and pulse-taking and palpitation 切) and treatment (such as herbal remedies, acupuncture, moxibustion, cupping, Qigong, massage, etc.) were aimed at identifying the types of imbalance and then restoring them to normal. From the various theories and diagnosis means which were the fundament of the whole TCM and existed for five thousands years up to today, Traditional Chinese Medicine was formed (18).

Over the long course of development of Chinese medicine, only few doctors stood out in history. *Bien Xu* (扁鵲) also called *Qin Yue Ren* (秦越人) was the most famous physician during the *Warring States* (戰國時期). His diagnostic methods were very similar to what we use today: listening to patient sounds, observing the patient spirit, inquiring, and taking palpitation/pulse. He specialized in obstetrics and became the first specialist. The development stages traced from records can be broken-down into broad categories of particular Chinese dynasties: the *Han dynasty* and before, the *three kingdoms* epoch, the *North-south division* epoch, the *Tang dynasty*, the *Song dynasty*, the *Yuan dynasty*, the *Ming dynasty*, the *Qing dynasty* and new China period.

With development of economy and culture of the Chinese nation, the theory of TCM is constantly improving. Each dynasty had its own outstanding representatives. The *Han dynasty* lived *Zhang Zhongjing* (張仲景), the greatest physicians in China. The *Tang dynasty* lived *Sun Simiao* (孫思邈) named Herbal King. In the *Jin-Yuan dynasty,* there had come into being four branches of TCM, named *Liu Wansu* (劉完素), *Zhang Zihe* (張子和), *Li Dongyuan* (李東垣) and *Zhu Danxi* (朱丹溪); each one with his characteristic and thesis significantly improved the theory of TCM. The *Ming dynasty* lived *Li Shizhen (*李時珍 1518-1593*)* and *Zhang Jingyue* (張景嶽), their achievements significantly promoted TCM development. The *Wen Bing school* (溫病學派) was developed in the *Qing dynasty*. TCM has taken shape uniquely with its own system and theory in practice, which develops its own way afterwards according to the theories.

Since the beginning of the 19th century, with western medicine entering and then developing rapidly in China, TCM development was affected severely; the status of TCM was between the devil and the deep sea. TCM was recognized as unscientific, feudal and had always been in the position of being investigated, examined, queried and reformed. TCM was like walking on thin ice since then (19).

After the establishment of the People's Republic of China, the Central Government affirms the policy to protect TCM. However, the development of TCM is far from going off smoothly. In the recent years, TCM has been in a difficult position again because there are queries that TCM is not scientific and should be abolished as Mr. Zhang Gong-yau (張功耀) proposed in his paper (20).

The Han dynasty (漢朝 206 B. C. – A. D.220)

Before the *Han Dynasty* (漢朝) (B.C. 206 – 219 A.D.) the methods that health practitioners used against illness was simply psychotherapy supported by operations

such as moxa, acupuncture, massage, and herbal concoctions without guidance by systematic theories. The *Han dynasty* (漢朝) (206 B. C. – A. D.220) was the most glorious period in Chinese medical history after a long-term exploring period. The *Han Dynasty* is sometimes recognized as the *Age of Science* in Chinese medical history. There were three important practitioners in this period who made great contributions to the Chinese medical development in Chinese history, these three great persons named *Cang Gong* (倉公), *Zhang Zhong-jing* (張仲景), and *Hua Tuo* (華佗) (21).

Cang Gong (倉公) was the first medical man in China to record clinical case. His most important contribution was to innovatively write down 25 medical cases during his practice. In therapies, the methods he used to treat diseases including herbal decoction, granular, gargle, herbal wine, pilular, as well as acupuncture, moxibustion, and cold compress. In diagnosis, *Cang Gong* greatly emphasized inspection and pulse taking. In his twenty-five case records, ten of them were diagnosed by pulse to judge whether the patients could survive or not. In 2000 years ago, it was very rare and valuable that *Cang Gong* had concerned the medical record. (22)

Zhang Zhong-jing (張仲景) was the most outstanding physician in ancient China, who was often spoken of as the Chinese "saint of medicine" and the "Hippocrates of China". He was especially known to later generations for his *Shanghan Zabing Lun* (Treatise on Febrile Diseases and Miscellaneous Diseases 傷寒雜病論), which contains over 100 effective formulas and many of them are still used today. The great book made a theoretical framework that led to hundreds of books analyzing, interpreting, and reforming it, it ranks with the *Nei Jing* (內經) in importance. With the advent of *Zhang Zhongjing*, a new time had come. Diseases were studied by a clinical system of

"*treating the patient according to syndrome differentiation* (辯症論治) " that was established for the first time and which is still widely applied nowadays (17).

Hua Tuo (華佗) (A.D. 145-208?) lived in the *Eastern Han dynasty* (東漢)(A.D. 25–220) He was born in *Anhui* province (安徽). He was the most famous for his expertise in surgery in ancient China (23). He was the first in the world to develop the use of anesthesia, and further the knowledge of anatomy. *Hua Tuo* treated patients with a combination of herbals and acupuncture therapy, he preferred simple methods, using a small number of acupuncture points and simple herbal formulas comprised of only a few herbs. He practiced *Qi Gong* (氣功) and taught the "*frolics of the five animals* (五禽戲)," which is still used today. The five animals are tiger, deer, bear, ape and crane. *Hua Tuo* inherited the medical achievements of past generations and strongly influenced the development of medicine during the *Wei Jin* (魏晉) and *Southern and Northern Dynasties* (南北朝) (24).

North South division epoch (南北朝 A.D. 425-590)

The first increase in recorded knowledge of Chinese material medica occurred in this period. The outstanding recorder was *Tao Hong-Jing* (陶弘景), a native of southern China (*Jiangsu Province*). He prepared two treatises which were *Transactions of Famous Physicians*, (*Ming-Yi Bie-Lu* 名醫別錄) and *A Commentary on Shen-nong's Herbal Classics* (*Shen-nong Ben-cao Jing-zhu* 神農本草經集注). *Tao Hong-Jing's* work was quoted repeatedly by *Li Shi-Zhen* (李時珍). *Tao Hong-Jing* (陶弘景) expounded *Shen-nong Herbs* on the one hand, and supplemented it with 365 kinds of medicinal substances on the other hand. His book doubled the list of known drugs to 730. It described the actions and uses of these drugs and classified them according to source into jades, stones, herbs, woods, fruits, vegetables, cereals, etc. This classification

method has become a standard in classification of Chinese medications for a thousand of years (25).

The Tang Dynasty (唐朝 617 - 906 A.D.)

The *Tang Dynasty* (唐朝) was a very prosperous period in Chinese history, the culture and medicine was spread widely. TCM education became standardized, which included nutrition, veterinary studies, specialization, and the standardized TCM formulas. Dispensaries were controlled and run by the government. The first herbal medicine school in China was established by the emperor in 629 A.D (26). This administration helped to decrease the number of fraudulent practitioners and herbal products on the market. It raised the standards and improved the quality of medical care for general population. During the *Tang Dynasty*, there was a great famous doctor named *Sun Simiao* (孫思邈). He was titled as China's King of Medicine (藥王, *Yaowang*) for his important contributions to Chinese medicine. *Sun* wrote two books - *Beiji Qian Jin Yao Fang* (備急千金要方"*Essential Recipes for Emergent Use Worth A Thousand Gold*") and *Qian Jin Yao Fang* (千金要方"*Supplement to the Formulas of a Thousand Gold Worth*") - these were both milestones in the history of Chinese medicine (27, 28).

The Song Dynasty (宋朝) (959-1278 A.D.)

In the *Song Dynasty* (宋朝), Chinese invented the printing techniques that made mass production of books possible. The first official edition of medicines of the dynasty was *Kai Bao Ben Cao* (開寶本草), which was the first printing published medicine book in China. Following the compilation of *Xin Xiu Ben Cao* (新修本草) in *Tang Dynasty*, *Kai Bao Ben Cao* (開寶本草) (20 volumes) was completed by high officials and practitioners such as *Liu Han* (劉翰), *Ma Zhi* (馬志), *Di Xu* (翟煦) and *Li Fang* (李

昉) under the auspices of the central government in the reign of *Kai Bao* (973 A.D.) in the *Song Dynasty*. The book, recording 984 kinds of substances, not only corrected the mistakes in *Xin Xiu Ben Cao*, but also added 134 kinds of substances to it (29). Unfortunately the original book was lost, but its main idea was recorded in *Zheng Lei Ben Cao* (證類本草). The first official organization, *The Bureau of Book Edition and Publication*, was established and many old books were published by the organization. Mass production allowed more books to survive. Many doctors could have more books to read and were better educated. Four Schools of Medical thought were developed based on the previous classics such as *Nei Jing* during the *Sung Dynasty* (30).

The Jin-Yuan dynasty (金元 A.D. 1206-1368)

There had come into being four branches of TCM in the *Jin-Yuan dynasty* (金元), each one with its characteristic and thesis, which significantly improved the theory of TCM. Under the prosperous and open environments, there were four outstanding figures. They were *Liu Wansu* (劉完素), *Zhang Zhihe* (張子和), *Li Dongyuan* (李東垣) and *Zhu Danxi* (朱丹溪). *Liu Wansu* (劉完素), who established "the school of cold-cool (寒涼派)", promoted the idea of using herbs of cooling nature to treat these conditions, which was the opposite direction of many of his predecessors using warming herbs. *Zhang Zhihe* (張子和) is known as the father of the "the school of purgation (攻下派)" of TCM, emphasizing the use of diaphoretics, emetics, and purgatives to attack the pathogen and drive it out of the body. *Li Dongyuan* (李東垣) established his famous thesis that most diseases were due to injury to the stomach/spleen system, which occurred as the result of intemperance in eating and drinking, overwork, and the seven excessive emotions, which was called "the school of nourishing the earth" (補土派). *Zhu Danxi* (朱丹溪) was good at temperance and use of tonic formulas, which was

called "the school of nourishing yin" (滋陰派)(31).

The Ming dynasty (明朝 A.D.1368-1644)

TCM was further improved in the *Ming dynasty* (明朝) (A.D.1368-1644). The most outstanding contribution in the *Ming Dynasty* was the publication of the *Compendium of Material Medica* by *Li Shizhen* (李時珍), the work improved theory and enlarged the scope of medical materials. *Li Shizhen* (李時珍) devoted about 30 years to his work named *Bencao Gangmu* (本草綱目), his works listed 1094 drugs from plants, 443 from animals, and 354 from minerals and other substances. Under each drug, the proper and popular names, source, form and general history were given, together with information from the literature and critical remarks, with details concerning collection, preparation, dosage and presentation, and a description of the nature and properties of each drug, with recipes and indications for use by the author. As early as the seventeenth century, it was translated successively into *Latin, Russian, Japanese, English, German* and *French*, and it spread all over the world (32).

Another outstanding representative in the *Ming Dynasty* was *Zhang Jingyue* (張景嶽). *Zhang* wrote many works on gynecology, pediatrics, surgery, pulse diagnosis, and an analysis of the *Nei Jing* (內經) called the *Lei Jing* (類經), which perfected the theory of TCM and made TCM developed rapidly (33,34).

The Qing dynasty (清朝 A.D.1644-1911)

Most medicinal plants used in traditional Chinese medicine, which were recorded in ancient herbals and pharmacopoeias, occurred before the *Qing dynasty* (清朝). Many new developments in various clinical fields had achieved in the *Qing dynasty*. In the prophase and metaphase of the *Qing dynasty*, medical specialists acquired prominent progress in medical theories and treatment of miscellaneous diseases.

The *Wen Bing* (溫病) (infectious febrile diseases) school was founding by *Wu Youke* (吳又可). The other outstanding representatives were *Ye Tianshi* (葉天士), *Xue xue* (薛雪), *Wu Jutong* (吳鞠通), and *Wang Mengying* (王孟英); and famous work in this period was monographs of The *Wen Bing Tiao Bian* (溫病條辨)(35).

Another important work named *Bencao Gangmu Shiyi* (本草綱目拾遺) (*Supplement to the Compendium of Materia Medica*) was published in 1765, which was written by *Zhao Xue-Min* (趙學敏). The book described 716 medical substances, which had not been included in *Li Shi-Zhen's Bencao Gangmu* (本草綱目). *Zhao's* contribution brought the total of the drugs recorded in formal literature at that time up to 2608. He collected and systemized the folk doctor *Zhao bo-Yun's* (趙柏雲) experiences in treating diseases in two books titled *Chuanya Neipian* (串雅內篇) (Internal Treatise on Folk Medicine) and *Chuanya Waipian* (串雅外篇) (Extra Treatise on Folk Medicine).(36, 37)

Recent Status of Traditional Chinese Medicine

TCM has changed over time although it is called traditional and it is very much a part of the modern world, especially in the recent years. In its 5,000 years of recorded history, TCM has evolved under many influences, including politics, economics, science, technology, and social and cultural changes. TCM came close to being officially prohibited and replaced by Western medicine at the beginning of last century. From 1911, China transformed from an empire to a republic, with western medicine entering and developing rapidly, TCM development was impacted severely. In February 1926, the KMD government raised a proposal "Abolish Traditional Chinese Medicine Act". Immediately a national wide strong opposition to the proposal was aroused. Ultimately, the proposal failed to approve the implementation, however, many kinds of discrimination, restriction on TCM made the development of TCM

very difficult (38).

Since 1949 the establishment of New China, the research and application of Chinese medicinal plants produced great changes (39, 40). Traditional Chinese medicine is encouraged and supported by China government (41). Since the 1980s, government has attempted to regulate drugs by law (42). In 1998, China streamlined its centralized regulatory processes for all medical products sold or manufactured in China, and *the State Food and Drug Administration* (國家食品藥品監督管理局 SFDA) was organized to formulate and implement relevant regulations (43). A new "*Administrative Measures for Drug Registration* (藥品註冊管理辦法)" was issued on February 28, 2005, and implemented on May 1, 2005. It is also applied to TCM medications.

The key steps of drug registration include:

- *Investigational New Drug applications:* a list of application documents and a sample of the drug should be submitted to the drug administration at the provincial level. Overseas drug manufacturers may apply direct to the SFDA.
- *Preclinical and clinical trials:* China introduced its first GCP guidelines in 1998; multiple designs, including single- or double-blinded randomized control are allowed. Trials in China are generally not placebo-based; instead, they compare the drug's performance with existing methods of treatment. In 2000, SFDA issued new rules stipulating that at least 50% of the work for Phase I-III clinical trials must be conducted by accredited National Clinical Trial Centers.
- *Quality Testing:* once clinical trials have been completed, the manufacturer should provide sufficient product samples, which have to be manufactured in three consecutive batches, for random tests.
- *Certification:* a registration certificate is issued once the quality test

process has been completed successfully.

- *Post-market Surveillance:* the level and quality of post-market surveillance activity is rising in China; all of the phase IV trials must be carried out at the same site as earlier trials.

In the past 60 years, a process of integration took place in China and it has become the accepted way of treatment. Today, Western medicine is playing a key role in modernizing traditional Chinese medicine in the methodology aspect. Taking modern scientific standards as references, we can find that TCM as a medical system has its own insufficiencies. In Chinese medicine, its folk categories, unique concepts, holistic view of the world and the human body, etc, makes it difficult to be understood by people from different cultures. The lack of quantification and qualification parameters of TCM makes it sound bizarre and unreliable to those who have no knowledge of Chinese culture. However, a medical system that has existed for thousands of years without broken in tradition and so far has served the Chinese people well must not be simply considered as unscientific and quackery. The integrative medicine has shown promising results giving full play to the strength of both and makes them supplement each other. It is more and more popular and welcome in China and even worldwide; and this is a clear testimony of its value and validity including safety and efficacy, and the increasing acceptance in the rest of the world (44) demonstrates the potential contribution of Chinese medicine to the good health of human being. However, only these evidences are insufficient, and further evidence-based clinical trials are required to demonstrate the efficacy and safety of TCM.

Traditional Chinese Medicine in Hong Kong

Although TCM was a major source of medical and health services for the Chinese people in Hong Kong until the 1950s, it was provided with little government support.

TCM was allowed to be used by the British government since it took over Hong Kong in 1842. The local peoples' customs should be respected according to the *Nanking Treaty* (南京條約), in which Hong Kong was ceded from China to Britain.

No public policy was issued to guide the use or practice of TCM. It was only in 1989 that a working party was set up to review and study the matter. The two drafts of the Basic Law, released in April 1988 and February 1989 respectively, contained an article that stated, after the handover, the Hong Kong government should formulate policies to develop both Chinese and Western medicine. This article (138) stayed in the final version of the Basic Law promulgated in April 1990. In 1997, a TCM division was set up. In 1999, the Chinese Medicine Council was established to oversee the regulation of TCM, which was Hong Kong's first TCM policy. In late 2000, TCM practitioners (herbalists, bonesetters, and acupuncturists) were invited to apply for registration. The first list of registered TCM practitioners was gazetted in November 2002 (45). Nowadays, the research, education and services of TCM in Hong Kong are developing fast but there are still obstacles barring further development (46).

1.2 Theories and treatment principles of Traditional Chinese Medicine

Traditional Chinese Medicine (TCM) is one of the oldest ancient systems of medicine that has been existed 5000 years (47, 48). The essential of TCM is based on a number of philosophical frameworks which include the theory of *Yin-yang* (陰陽), the *Five Elements* (五行), the human body Meridian system (經絡), *Zang Fu* (臟腑) organ theory, and others. Diagnosis and treatment are performed in compliance with these concepts. (49).

Traditional Chinese medicine had focused on the observable natural laws of the universe and humanity's place in the universe. Optimum health results from allowing

the spontaneous process of change to bring one closer to balance (50). If there is no change (stagnation), or too much change (catastrophism), balance is broken and illnesses can result. TCM is a holistic approach, and emphasizes the importance of keeping all the structures functioning harmoniously. A holistic approach is always used when addressing imbalance (51).

Concept of Yin-Yang (陰陽) *and Five elements* (五行)

The theory of *Yin—Yang* and *Five Elements* are the core theories of TCM, which believed that the world was material, and the material world was multiplied and developed by *Yin—Yang* and five basic matters including wood, fire, earth, metal and water, which were indispensable elements that formed world (**Figure 1-1**) · The five kinds of matters were interdependent and counter balance each other, situated in continuous movement and change.

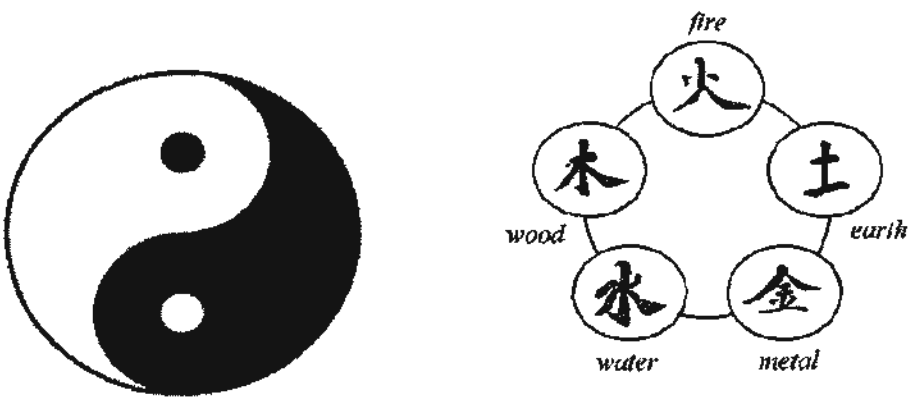


Figure 1-1 Yin-Yang and Five elements

The theory of *Yin-Yang* and *Five Elements* were applied to explain origin of mankind, physiological phenomenon, pathological changes and to guide clinical diagnosis, prevention and treatment. They have become an important part in Chinese medicinal

theory (52. 53, 54, 55).

Huangdi Neijing (黃帝內經) states " The *yin* and *yang* are the law of the heaven and the earth, the rule of everything, the parents of variation, the root of life and death, and the locus of power of the universe" (56). It could be said that all TCM physiology, pathology, and treatment can be explained by the relationship between the forces of *yin* and *yang*. Disease patterns may be described as being "Excess *Yin*"(陰盛) or "Excess *Yang*,"(陽盛)"Deficient *Yin*" (陰虛)or Deficient *Yang*."(陽虛) (57).

According to *Huangdi Neijing* (黃帝內經), there are four relationships of *yin* and *yang* (58, 59, 60):

- The divisibility of *yin* and *yang*: in TCM, any object can be divided into two attributes of *yin* and *yang* in opposition
- The interdependence of *yin* and *yang*: *yin* and *yang* are mutually indispensable and engendering
- The interrestraint of *yin* and *yang*: *yin* and *yang* are mutually constraining. The weakness of either *yin* or *yang* will lead to the preponderance of the other (loss of constraint)
- The mutual transformation of *yin* and *yang*: any two of the aspects of *yin* and *yang* can be converted into its opposite when it develops to a certain stage

Similar to the theory of *yin-yang*, the theory of *five elements* was an ancient philosophical concept used to explain the composition and phenomena of the physical universe. Ancient philosophers believed that nature consist of five basic elements: wood, fire, earth, metal and water, which are interdependent and counterbalance each other in a situation of continuous movement and change. They thought that the

development and change of every thing and phenomenon were the results of evolution and interaction of *Five Elements* in universe (61).

The *Five Elements* are attributed and classified as: Generation, restriction, subjugation and reverse restriction of *five elements*. According to the theory, the *five elements* are in constant move and change, and the interdependence and mutual restraint of the *five elements* explain the complex connection between material objects as well as the unity between the human body and the natural world (62).(**Figure 1-2**)

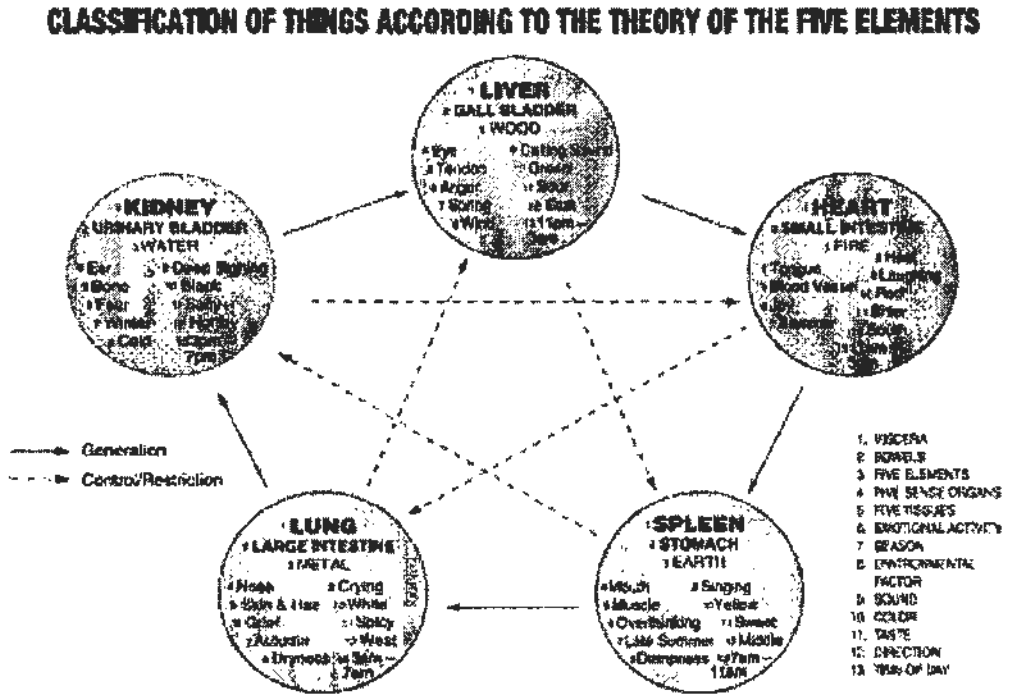


Figure 1-2 Classification of things according to the theory of the *five elements* [from TCM World Foundation] (63)

ZHENG (syndrome 症) in Traditional Chinese Medicine vs. DISEASE(病) in Western Medicine

Traditional Chinese medicine uses ZHENG as the key pathological principle to guide the applications of Chinese herbal medicine. All diagnostic and therapeutic methods in TCM are based on the differentiation of ZHENG (辨症), and this concept has been

used for thousands of years in China (64).

ZHENG is seen as the TCM theoretical abstraction of the symptom profiles of a disease, not simply an assemblage of disease symptoms. It is quite different from DISEASE in Western Medicine. For example, patients suffering from the same disease may be categorized into different ZHENG, whereas different diseases may be categorized as the same ZHENG. The 'Cold' ZHENG (寒症) and 'Hot' ZHENG (熱症) are the two key statuses of ZHENG, which therapeutically direct the use of Chinese herbs in TCM. The terms of major symptom profiles for Cold and Hot ZHENG are listed in **Table 1-1** (65).

Table 1-1 Major symptom profile terms of Cold ZHENG and Hot ZHENG

Subjects	Terms (keywords)
Cold-ZHENG-related symptom profile terms	Cold (chill, coldness); cold pain; tastelessness; clear abundant urine (clear urine in large amounts); loose stool; pale tongue; white fur (white moss); tight pulse (stringy pulse)
Hot-ZHENG-related symptom profile terms	Fever; heat (hot); diaphoresis; flushed face; burning pain; deep-coloured urine; red eyes; thirst; desire for drinking; constipation; red tongue; dry tongue; thin fur (thin moss); yellow fur (yellow moss); rapid pulse

The nature of a disease usually includes etiology, pathology and the location of the disease. Modern medicine is trying to identify the specificity of the cause, pathology and location, and as a result, the therapeutic approach is targeting on the specificity. New drugs in modern medicine are developed from strictly designed scientific pharmacological tests that are targeting on the specificity. It is believed that the non-specificity sometimes could influence or change the process of morbidity; and only targeting the specificity is not enough to stop the progress of morbidity (66).

ZHENG mainly refers to the non-specificity that is only obtained from symptoms and signs by asking, watching and feeling since there are no modern diagnostic

instruments. Chinese herbal medicine, based on the ZHENG which is taken as an outcome of differentiation of symptoms and signs, targets to the non-specificity and part of the specificity. The effect of herbal medicine is not so good in curing a disease with specific signs. However, the effect of herbal medicine is better in treating some cases when non-specificity decides the process of a disease (67).

Using the TCM ZHENG theory, different diseases may be treated by the same therapeutic approach if they show the same ZHENG. Similarly, the same disease may be treated by different therapeutic approaches if the disease shows different ZHENG. It is common in TCM that one kind of disease is treated with different therapies. As mentioned above, ZHENG is the outcome of differentiation of symptoms and primary signs obtained by getting from watching (tongue watching) and feeling (pulse feeling). Definitely ZHENG is not so accurate. For example gastritis and stomach cancer could show similar symptoms and primary signs, suggesting that they could be differentiated as the same ZHENG in TCM, and treated by the same TCM approach. Combining the differentiation of ZHENG with diagnosis of DISEASE, which is combining herbal medicine mainly targeting non-specificity with modern drugs targeting the specificity, one would achieve better therapeutic effect. This is currently called integrated medicine.

Treatment principles of TCM

The principles of TCM treatment can be summarized as followings:

1) Prevention as Priority (治未病)

   Determine the root cause is the overall guiding rule in treatment of diseases in Chinese medicine. The most important is to take precautions before disease strikes. There are many formulas and methods to help enhance the immune system, and most TCM formulas have the power of prevention. During SARS

crisis in 2003, Institute of Chinese Medicine CUHK developed a herbal formula based on ancient classic formulae, and prepared as herbal tea distributed to healthcare workers in hospitals. The results suggest that there is a good potential to prevent the spread of SARS (68).

2) Balance as the Ideal State of Health (養身)

In Chinese concept, everything is connected in a relationship of cooperation and coordination. For the human body, when all are maintained in a relative equilibrium and balance, ideal health prevails. If there is anything wrong in the body, equilibrium is broken. The health status may be affected. It is the mission of Chinese medicine to address this balance. What is more, in reestablishing this balance, efforts must be taken to avoid creating any new imbalance.

3) Holistic considerations and Individualized Treatment

In prevention, diagnosis and treatment, everything, including the season and local conditions, physical and psychological environment, the patient's age, gender and general physical conditions, family history, etc, all have to be considered. The holistic approach of TCM has contributed to the classification of the human body into functional systems rather than particular organs in anatomical sense. This is one of the important differences from western medicine.

4) Strengthen the Immune System and Eliminate Pathogenic Factors

The body's ability to defend itself is most important. In case of weak defense, the emphasis has to be on enhancing the immune system in order to eliminate pathogens (固本培元).

5) Differentiation of Root Cause from Symptoms and Determination of Acuteness of the Disease

To treat the root cause (治本) instead of the symptoms (治標) is always the principal aim of Chinese medicine, but in treatment considerations are given to

the actual circumstances.

6) Straight Treatment and Paradoxical Treatment

Straight treatment is to treat cold with heat and heat with cold, supplement in case of inadequacy (vacuity), and discharge in case of excess. These are the normal ways of treatment. However, when symptoms do not reflect the root cause, the opposite is called for. For instance cold symptoms may be the result of extreme heat, and in such case the right way is to use cold method.

1.3 Important ancient classic documents of Traditional Chinese Medicine

A great deal of knowledge in the form of ancient books and literatures were accumulated through numerous TCM practices and theoretical researches in thousands of years. The domestic collection of the ancient books about TCM published before *Xinhai Revolution* (辛亥革命 1911) reached 130,000 volumes (69). The followings are some of the most important ancient TCM classics.

The Medical Classic of the *Yellow Emperor* 《黃帝內經》

The *Medical Classic of the Yellow Emperor* is one of the earliest books found in the Chinese history. Many historians and physicians throughout Chinese history studied and verified that the major contents of The *Medical Classic of the Yellow Emperor* had appeared in the *Warring States Period* (戰國時期 475 BC -- 221 BC). It had been added during the *Qin Dynasty* (221 BC -- 206 BC) and *Han Dynasty* (206 BC -- AD 24). It is one of the four classic works of Traditional Chinese Medicine. It established the systematic structure of the theoretical system of TCM. This theoretical system has formed the basis of development of TCM since its establishment (70). The fundamental principle of The *Medical Classic of the Yellow Emperor* is the theory of *Five Elements*. The integral idea that man and nature are mutually corresponding was

established. This is a unique feature of TCM that differs from almost all other traditional medicines.

The *Medical Classic of the Yellow Emperor* presents broad concepts and is often brief with details. There is much to be gained by understanding it from a perspective of openness as it generously shares the wisdom imparted from an ancient tradition benefiting the health and lives of humankind. Chinese medicine has changed little from the time The *Medical Classic of the Yellow Emperor* was written. Its natural therapies and preventive approaches are ever as effective and even more pertinent in today's drug-oriented medical climate. While showing us that from the microcosm of human life we may learn the vast and profound realities of the macrocosm, it offers a heartfelt and viable approach in the perception and treatment of illness (71, 72).

Shennong Bencao Jing 《神農本草經》

Shennong Bencao Jing (神農本草經) was the earliest record of Chinese herbal medicine (around 2800 BC), which was published during the *Eastern Han Dynasty* (1st century BC) (73. 74). This book classified 365 herbal materials into three categories: Superior (120, acted as Jun 君), Intermediate grade (120, acted as Chen 臣) and Inferior herbs (125, acted as Zuo 佐 and Shi 使). Superior grade materials have good health promoting (or tonic) effects and low toxicity. Frequent consumption is recommended for the promotion of health and longevity. One of the most famous herbs in this category is ginseng (Panax ginseng). The intermediate grade materials are mostly medicinal agents with therapeutic effects but they may produce toxic side effects if the materials were used inappropriately. The inferior grade materials are mostly toxic substances and should be used with caution to treat some diseases. This grading system reflects the traditional Chinese concept that promotion of health is

superior to disease therapy (75). Among them, 252 are plants, 67 are animals and 46 are minerals. Most of the herbs cited in the *Shennong Bencao Jing* are still found in standard herbal textbooks and in herbal pharmacies today (76).

Shennong Bencao Jing established basic Chinese herbal theory including four *Qi* 四氣 (cold, hot, warm and cool); *five flavors* 五味 (sour, salty, sweet, bitter, and pungent); formula structure (chief, deputy, envoy, and assistant); prescription principles; decoction methods; and dosage timing for different disorders. The indications of the 365 herbs cover more than 170 diseases. (77, 78)

Shanghan Lun《傷寒論》

Treatise on Febrile Diseases (*Shanghan Zabing Lun*《傷寒雜病論》) was written by *Zhang Zhongjing* (張仲景), the sage of Chinese medicine in the *Eastern Han Dynasty*. *Zhang Zhongjing's Treatise on Febrile Diseases* may be the most influential work of Chinese clinical medicine in history. This is also the seminal text combining theory and practice. *Treatments based on the method of pattern differentiation* (Bian Zheng Lun Zhi 辨證論治) presented in the book were followed by generations of Chinese physicians and became the standard of practice in traditional Chinese medicine. He innovated the *Three Yin-Three Yang* diagnosis and treatment system. The *six-meridian pattern differentiation*(六經辨證) (Greater *Yang*, Brighter *Yang*, Lesser *Yang*, Greater *Yin*, Lesser *Yin* and Terminal *Yin*) is a classic approach in Chinese medicine diagnostics. This is a classical application of pattern differentiation with eight principles (八綱)(*Yin/Yang*, *Exterior/Interior*, *Deficiency/Excess and Cold/Heat*) (79).

Exterior and interior conditions are prioritized with the eight general treatment methods (sweating, vomiting, draining, harmonizing, warming, clearing, reducing,

tonifying). Although the book was written for febrile diseases, it is well beyond that through the time. The 113 formulas presented in this text are known as classic prescriptions dealing with diseases from the common cold to liver cancer.

Assimilating from previous medicinal literature, and collecting many prescriptions elsewhere, *Zhang* finally wrote the medical masterpiece. The book contains six parts, which correspond to the six pairs of meridians. It is significant because it discusses diagnosis and treatment methods based on an assessment of the symptoms of different pathological conditions. Later in the *Song dynasty*, his book was rewritten and divided into two books called *Shanghanlun* 《傷寒論》(*Treatise on Febrile Diseases*) and *Jinkui Yaolue* 《金櫃要略》(*Synopsis of the Golden Cabinet*).

The Synopsis of the Golden Cabinet 《金櫃要略》 is the first systematic medical text for internal diseases, gynecological disorders, and dermatological conditions. It laid out the principles of pattern differentiation and prescriptions in a coherent way. The book consists of three volumes, and includes 262 formulas. Most of these formulas have been in use since they were developed. Because this book contains the most basic formulas used in clinical applications, it is called "*the grandfather of prescription books* (方書之祖)."Formulas such as *Bai Hu Tang* 白虎湯 for summer heat, *Yin Chen Hao Tang* 茵陳蒿湯 for jaundice, *Shen Qi Wan* 腎氣丸 for diabetes, *Gua Lou Xie Bai Tang* 栝蔞薤白湯 for chest Bi-syndrome, and *Huang Tu Tang* 黃土湯 for blood disorders are time-tested and clinically reliable. Other contributions from this text include preventive treatment, the trilogy theory of diseases, creating a variety of herbal forms, laying the foundation of TCM internal medicine and TCM gynecology. (80).

***Bencao Gangmu* (本草綱目) (ca. 1592—1596)**

Grand Materia Medica (本草綱目 *Ben Cao Gang Mu*) is China's most celebrated

pre-modern herbal book. Translated into many languages, it contains 1892 entries, including 1173 from plants, 444 from animals and 275 from minerals. Darwin referred to the *Grand Materia Medica* as China's encyclopedia. It contains a summary of the herbal experience and knowledge accumulated before the 17th century. A complete classification system of herbs is established and 11,000 formulas are included: descriptions, corrections, explanations, flavors, indications, contraindications, functions, graphics, associated formulas are detailed in this mastery herbal book.

This work is probably the best known of all Chinese classic herbals. It was compiled by *Li Shi-zhen* (李時珍) who is considered as China's greatest herbalist. In this work, the number of Chinese materia medica (CMM) items described had increased from 365 recorded in *Shennong Bencao Jing* (神農本草經) to 1800 plus 11100 prescriptions. The *Bencao Gangmu* is one of the most frequently mentioned books in the Chinese herbal tradition, rivaled only by the *Shanghan Lun* (傷寒論).

The *Bencao Gangmu* is considered uniquely valuable for several reasons (81, 82, 83).

1) Covered a huge number of diverse materials

It presented a huge number of diverse materials: 1,892 medicinal substances (1,094 from plants; 444 from animals, and 275 from mineral sources), including 374 new items.

2) Formulation strategies

Li collected virtually all the prescriptions that had been handed down over the centuries and then presented over 11,000 formulas: about 2,000 of these were well known from other medical works, but over 8,000 were collected by *Li* from contemporary doctors and rare texts. With regard to ginseng, he recorded 77 prescriptions that included the herb, of which only 9 were ancient ones (including *Si*

Junzi Tang (四君子湯), *Four Major Herbs Combination*). Further, he explained the principles of the formulations (84).

3) Unique organization

As important as the breadth of the collection was its unique organization. *Li* was able to categorize the medicinal materials into more logical groupings than had been achieved ever before. The groupings came close to the binomial system introduced by Carl Linnaeus during the 18th Century (85). Charles Darwin (86), in working out his theory of evolution, is reported to have quoted from the *Bencao Gangmu*, which had such detailed information about the variations of plants and animals that it helped fill out the theory.

4) Historical record

The *Bencao Gangmu* is appreciated as a historical record because *Li* quoted from 952 previous authors. In fact, he also provided an extensive bibliography, listing 277 books on medical subjects and herbs and 591 other texts, such as literary classics and historical works. Joseph Needham, the famous British historian who spent most of his adult life on the multi-volume work, Science and Civilization in China, declared that the greatest scientific achievement in the *Ming Dynasty* was the *Bencao Gangmu* (87.).

In the long course of Chinese history, Chinese medicines developed from a mythical medicine to a systematic medicine. Although Chinese medicinal drugs had been recompiled many times, the fundamental contents remained the same. This is because the fact that the evaluation of a drug could only be recorded in the hands of famous and well-experienced physicians. Many of their famous prescriptions were handed down from generation to generation with minor alterations. In other words, these herbal agents had to be screened through the clinical experiences of many ages to be

effective, with not a few being remarkably efficient. The process of trial and error gradually eliminated the worthless or less efficient ones. At present there are about 600 effective agents in common use, about 100 of which are at the top of the list for scientific studies in pharmacology, botanical classification, chemical analysis and clinical evaluation (88).

1.4 Philosophical Differences between Modern Medicine and Chinese Medicine

TCM and Western medicine (WM) or modern medicine have different viewpoints about etiology and pathology and thus different diagnostic methodologies (67). The largest difference in philosophy between Western and Chinese medicine is how to view the body.

Modern Western therapy is not concerned with an integrated analysis of the whole system of the human being. Traditional Chinese Medicine views the body as an ecosystem. (89). Western medicine treats diseases and ailments that are visible, structural, and mechanical in nature through the use of chemical drugs and surgical operations. Traditional Chinese Medicine does not treat structural changes. It dedicates itself to the treatment of physiological and functional imbalances that are invisible. Traditional Chinese Medicine dated back nearly 5000years began with the observation of man and his relationship to nature. The philosophy of Traditional Chinese Medicine comes from Taoist teachings and is one of integration and balance.

Western medicine looks upon the body as being very mechanistic. After the Middle Ages, the great philosophers separated the union of man, nature and heaven introducing analytical reasoning. Descartes' statement that man's physical body is separate from his soul began the scientific study of the body through autopsy and the scientific method. Western medicine then began to fix the ailing "part" or diseased

system without viewing the entire person as a whole.

Western medicine in general acts upon the definite target (single target) by pure chemicals. TCM works more on the restore of body balance by acting on the multiple targets. Chinese medicine is very good at treating the diseases that Western medicine considers "idiopathic" that means the cause is unknown, such as Fibromyalgia, Irritable Bowel Syndrome, and Chronic Fatigue Syndrome. In Chinese medicine the cause is simply a stagnation of the flow of *Qi* energy due to a variety of factors. The fact is, the cause may be not physical, but the symptoms are. Western medicine can see and measure certain changes in the body's chemistry and functional activities, but cannot act upon these changes for lack of understanding of their cause. The symptoms are too divergent and unrelated from a mechanistic viewpoint (90).

The healing system of Traditional Chinese Medicine (TCM) believes that diseases are the result of underlying malfunctions of the organs or imbalance of the *yin* and *yang* of the body. When disease develops, regardless of what it is and where it is located, the entire body is affected. Thus, treatment should be directed toward the cause of the disease and the whole person. However, Western medicine believes that a disease is an isolated entity in the body and treatment is usually directed toward the symptoms only.

The TCM approach views each patient and each ailment as unique. The cause of the disease may be different even if two people are suffering from the same ailment and is thus treated differently. This is more effective than the conventional Western approach of providing identical drug therapy to all persons having similar symptoms.

TCM therapy is primarily empirical and holistic, while Western drug therapy is experimental science-based and targets specific symptoms and obvious disease-causative agents. TCM emphasizes prevention and restoring balance that

often are intangible, while Western medicine attempts to treat what is scientifically obvious or tangible. Western drugs are mostly single chemicals that are readily identified and quantified, TCM are multicomponent therapeutics whose strength is due to their unique combinations around which this whole therapeutic system is based.

Table 1-2 Differences in cultural priorities---Western science and Traditional Chinese Medicine (50)

	TCM culture	**WM culture**
Philosophical	Man-nature unity Evaluational Metaphorical Balancing Analyze whole changes - zhang essence, qi	Conquest of nature Factual Proof by evidence Experimental Analyze structural states - specific organs & cells
Instrumental	Philosophical Use 8 states perspective for therapy	Scientific Use scientific technology to treat disease
Inquisitive	Functional observation - knowing from external to internal Inductive Perspective work up	Structural behavior - point analysis Deductive Factual logic for hypothesis therapy
Preferential	Perfectionistic	Realistic

Based on the holistic approach, TCM plays a unique role in the development of life science and medicine. With the dramatic increase in the prevalence of chronic conditions, the chemical medicines cannot meet the requirements of health maintenance, disease prevention, and treatment. Human health demands the large-scale development and application of natural medicines, to which TCM experiences and knowledge can make a great contribution. The ever-increasing use of

Chinese medicine (Chinese herbal medicine and acupuncture) worldwide is a good indication of the public interest in TCM (91, 92).

The therapeutic principles and goals of traditional Chinese medicine (TCM) are different from those in Western medicine. TCM does not focus only on the disease defined by specific pathological changes, but instead concentrates on the overall functional state of the patient. However, it remains unclear which parameters represent this general functional state. TCM has a complete system of classifying functional states but its unique and seemingly inaccessible language forms a mysterious veil over its therapeutic philosophy.

1.5 Efficacy of TCM from historical reflection

Traditional Chinese Medicine had played a very important role in the prosperity of Chinese population. In ancient China, innumerable and valuable experiences have been recorded in the course of fighting epidemics, which were the most serious threat to the survival of Chinese people. From 171 to 185 AD, there were several great pandemics happened in China. Hundreds and thousands of people suffered from attacks and lost their lives. For this reason, Chinese medical practitioners in ancient China were already concerned with epidemics, and one of the most important works was '*Exogenous Febrile Diseases*' (EFD) 《傷寒論》. Since the *Han Dynasty* (206 BC to AD 220) nearly every medical book has emphasized the importance of the study of epidemics. There must be around 250 texts written on epidemics from ancient China. Many Chinese medicine practitioners devoted their whole lives to the study of methods of prevention and treatment of pandemics.

Ancient healers in China recognized two characteristics of epidemics: high

infectiousness and uniform presentation. In *Huangdi Neijing* (黃帝內經), the following is recorded: "when epidemics came, almost everyone would be infected and the symptoms were similar, whatever the ages". Later, *Xu Chen* (許慎) described in his book *Shuowen Jiezi* (說文解字) in 121 AD that, "so called epidemics mean everybody infected". Some other healers observed that the spread of an epidemic was related to atmospheres and seasons.

Recorded epidemics in ancient China were certainly plentiful. From *Shi Ji* (史記 historical records) from 369 BC to the later stage of the *Ming Dynasty* (1368—1644), a total of 238 epidemics were recorded, among which 95 were officially entered by government authorities. The descriptions for these great pandemics included phrases such as: "the dead persons were innumerable", "for most families nearly every member died", "even a whole village extinct", "every family suffered from the pain of lost family members" (93).

The most notable epidemic occurred in 1918. Nearly one-third of Americans were infected, (94) and their life expectancy for this time was decreased by 10—12 years because of the epidemic. (95,96). In the 1918 episode no report from China carried similar messages. Influenza did spread widely in China in 1918—1919, but it was relatively mild and less lethal than elsewhere in the world, despite the generally poor levels of health at that time. Outside China, the mortality of influenza was very high. (94, 97, 98, 99, 100) During those years, modern medicine was undergoing its early phase of development, and a high mortality was expected in cases of viral infections affecting the lungs. How did patients in China manage to survive better? The records indicate that influenza was widespread in China in 1918 to 1919 (101,102, 103), but although severe in some parts, it was mild in many places compared with elsewhere in

the world. One explanation is that the 1918—19 influenza virus, or a closely related precursor, originated in China, so that many Chinese had had prior exposure and hence some immunity was obtained. However, after tracing all known outbreaks of respiratory disease in China, Jordan (104) concluded that none of them "could be reasonably regarded as the true forerunner" of the pandemic. We think the likely explanation is that traditional Chinese medicine might have played an important role. Why was China spared from a more serious impact of the 1918 influenza pandemic? It is well known that China was an undeveloped and closed-door country at that time, and it was not likely that China's general population used Western medicine as the main means of disease treatment. Traditional Chinese medicine would have been the only form of treatment that the general public relied on. According to records an anti-pandemic companie was performed in *Chengde County* in *Hebei Province* on October 23, 1918. The local government announced "recently the county was troubled by epidemics which spread widely in a high speed . . . When a person became infected, the other family members soon became infected too. If no prompt actions were taken, the situation might be out of control." and a series of measures were taken to control the pandemics "houses should be sprayed with limewater or lime powder, and rhubarb and Atractylodes rhizome should be burned to disinfect the air". For prevention, "villagers were advised to drink more soup prepared with powdered mung bean and rock sugar, several times a day." For those who had been infected, "more than 5000 doses of herbal formula were distributed to the families." These measures were very efficacious. The recovery rate was 97.1% and mortality rate was 2.4% (105). Compare this with San Francisco in the USA, the mortality there was as high as 8.98% (106).

Each generation of medical specialists had tried their best to explore the reasons for

the prevention and treatment of the epidemics as previously mentioned. Efficacious theories, therapeutic methods and herbal formulations were developed. Besides the herbal preparation therapies, Chinese medical practitioners at the end of the *Ming Dynasty* and early *Qing Dynasty* (1644—1911) also developed preventive vaccination techniques for the prevention of smallpox. (107,108) Historical records suggest that ancient Chinese healers had recognized that there were other useful means for the prevention and treatment of epidemic diseases apart from herbal medicines. Although the treatment of epidemics affecting the respiratory tract was not attempted with methods other than herbal medicines, Chinese healers did attempt to give early and preventive treatment during an epidemic attack (105).

According to the theory of traditional Chinese medicine, influenza is classified as *Wen Bing* (溫病 epidemic febrile disease) or *Shanghan* (傷寒 febrile disease).Well-known examples of effective herbal formulations include *mahuang xingren shigao decoction* (麻杏石甘湯) created by *Zhang Zhong-jing* (張仲景) (*Han Dynasty*), *Sang Ju Yin* (桑菊飲) and *Yin Qiao Sam* (銀翹散) (吳鞠通 Wu Ju Tong, *Qing Dynasty*),(109). and *Yu Pin Feng San* (玉屏風散) (朱震亨 *Zhu Zheng Hen, Yuan Dynasty*).(110)

The use of Chinese medicine in anti-epidemic therapy has stood the test of time, is still trusted and maintains its popularity. Nevertheless in this modern era when all treatment medications need scientific proof of efficacy and explanations as to their mode of action, the herbal formulae need to be re-investigated. If they are effective against early influenza, how do they work? Is it an antiviral mechanism, or an immuno-modulating mechanism to boost resistance? Since literature research on the impact of Spanish influenza does indicate that the Chinese people in China survived much better than people in the USA and Europe, and Chinese people during that period relied invariably on Chinese herbal medicine as the only source of treatment and prevention, we find ourselves encouraged to go further in our attempts to

understand more about Chinese medicine and influenza.

As most of the ancient records are considered empirical experiences, large and well-designed randomized controlled trials (RCTs) on long-term major outcome should be performed. In fact many RCTs have been conducted in China to evaluate the effectiveness of traditional Chinese medicine with encouraging results, but the methodological quality needs to be improved (111). This thesis would like to explore a practical and appropriate methodology for the development of Traditional Chinese Medicine.

Five thousand years of Chinese medicine history is the course of research methodology exploration. From the tasting of herbs by *Shen Nong* to the treatment based on syndrome differentiation proposed by *Zhang Zhong-jing*, all of these were intended to use different approach from different perspective to understand and explore Chinese medicine. Along with social progress and scientific and technological development, research methods of traditional Chinese medicine also need to improve. Since the "syndrome differentiation of six channels theory" proposed by *Zhang Zhong-jing* in the *Han Dynasty,* for thousands of years the development of traditional Chinese medicine followed along this path, and gradually detached from the important basis of morphology. During the same period of *Zhang Zhong-jing* (150-219AD), the Western physician Galen (129-200AD) established experimental studies and morphological studies, and initiated the modern medical research methods. From the foundation, Western medicine got great success and achieved rapid development. This was an important dividing line between East and West medicine. Since then, Chinese medicine developed a set of rigid, obscure theories, groped in darkness, gradually far away from modern medicine. The methodology of Chinese medicine research needs improvement; the traditional research and development method of Chinese medicine will only make the road get narrower. Evidence-based

study seems to be the way to go for Chinese medicine research. However, Chinese medicine and Western medicine are two completely different systems. Is it suitable to completely adopt the methods of Western medicine research for the research of Chinese medicine? What kind of research method is suitable for the development of Chinese medicine? This thesis is intended to look for the solutions. Research of Chinese medicine is facing a series of methodological difficulties, such as quality control, efficacy and safety studies, clinical research design and implementation, the synergistic effects between traditional Chinese medicine and western medicine, the material basis of efficacy of Chinese medicine and the action mode, etc., these issues will be elaborated in subsequent chapters.

Chapter 2

Current Research Areas in Traditional Chinese Medicine

Evidence based medical research has been successfully applied in modern medicine. However, research in traditional Chinese medicine is still in its mechanism centred approach and has been dominated by studies of basic and intermediate mechanisms. Though tremendous efforts have been made, and despite occasional successes, the nature of disease in traditional Chinese medicine such as action model, metabolism, clinical efficacy and long-term safety —have not been satisfactorily answered. This chapter reviews the current approaches used in Chinese medicine research.

2.1 Classic theory and treatment principles

The recognition of structure and function of the human-body in Traditional Chinese medical science is insufficient. Many concepts of traditional Chinese medicine is overriding on the anatomy, it is actually ignorant of the organ anatomy of human body. TCM emphasizes function and looks down on the actual entities, emphasizes the connections of the whole body and underestimates the local exists, so that anatomy is no longer taken seriously. "*Existing inside means shaping outside*" ("有諸內必形諸外"), detecting internal changes by observing external manifestations becomes the primary subject of Traditional Chinese medical research and logic replace the anatomic observation, leading people to a illusory realm, mixing structure with pathology. There is an invisible, intangible "*Qi*", it prevails and flows all over the body through "meridians", but without anatomical realm. The entire Traditional

Chinese medicine is virtually setting up on changes of function without human body structure. Due to lack of structural concept, the micro-scientific laboratory parameters such as cells, viruses, bacteria, hormones, neurotransmitters, genes were not adopted in Traditional Chinese Medicine researches. West medical sciences have got a lot of benefits from the morphology; while Traditional Chinese medical science has to suffer a lot because of ignoring the morphology. Apart from the painful previous experiences, many present TCM or integrated projects that last a long time and invested with a lot of money are still rejected by mainstream medicine.

TCM experts often respect case reports and anecdotal reports. However, the evidences that are only relying on the case reports and / or anecdotal reports are not accepted by the mainstream medicine. It is critical to use modern evaluation methods in assessing the efficacy of Traditional Chinese medicine.

2.1.1 Holistic concept (整體觀) in Traditional Chinese Medicine and Reductionism (還原論) in modern medicine

TCM is rooted in Chinese cultures; it has a deep relationship with Chinese philosophies. TCM concerns the integrity and holism of the human body and its interrelationship with the nature. The theory of TCM recognizes an individual as a whole, and this characteristic of consideration endows its holistic medical pattern. Both man and nature are originated from the same thing.

The emphasis of Chinese medicine is on the patient rather than the disease. Chronic and age-related diseases (such as antioxidant and antiaging, blood pressure-lowering, hypolipidemic, blood sugar-lowering, anti-allergic, and anti-arthritic effects), disease prevention and health maintenance are attractive targets, which are more suitable for

dealing with by using Chinese medicine.

In contrast to the holistic approach of Traditional Chinese medicines, modern science is skewed towards reductionism, breaking down organisms to the cellular, subcellular and even molecular levels. Modern medicine focuses on parts and often suppression of symptoms, opting for immediate results rather than long-term fortification of the whole body. The holistic and systematic ideas of TCM are essentially different from the thinking modes based on Reductionism in modern medicine.

Consequently, unlike modern medicine TCM does not focus on a single target but on multiple targets and focus on the balance of the body in a holistic manner. Based on the methodology of holism, TCM plays a unique role in advancing the development of life science and medicine with the dramatic increase in the prevalence of chronic conditions that chemical medicines cannot totally satisfy the needs of health maintenance, disease prevention, and treatment.

2.1.2 Treatment based upon syndrome differentiation (辨症論治)

Syndromes in Chinese Traditional medicine is called *Zhenghou* (症候). *Zheng* (症) means evidence, *Hou* (候) means phenomena or manifestation, which consist of a number of special symptoms and signs as a result of human body responses to exogenous and endogenous pathological agents.

The significance of syndrome differentiation in TCM was stated as early as in *Tang Dynasty* as "The same internal disease may be represented by different external symptoms and signs, and different internal diseases may have the same external manifestations and vice versa".

Guided by the "*yin-yang* and *five-element* theories"(陰陽五行理論) and supported by the "viscera-state and meridian doctrines"(臟腑經絡學說), traditional Chinese medicine (TCM) elucidates the kinetic laws of human birth, aging, disease and death, and treats

illnesses in the light of "syndrome differentiation".

Syndrome differentiation, a crucial step in clinical practice of TCM, is a comprehensive analysis of external manifestations of the human body based on TCM theories, and reflects physio-pathological changes in the human body (from the TCM viewpoint). Treatment based on syndrome differentiation is the core of TCM therapy.

The component parts of the human body are inseparable and are functionally coordinative and mutually beneficial while affecting each other pathologically. TCM adheres to the basic principle of treatment based on differentiation of symptoms and signs, treats the same disease by different methods and different diseases by the same method, and advocates individualized treatment, which vividly reflects the essence of TCM treatment.

2.1.3 Differentiating Syndromes (*Bian Zheng* 辨證) versus Orthodox Modern Diagnoses (*Bian-Bing* 辨病)

During the process of TCM efficacy evaluation, the diagnostic criteria for subject selection are based on "ZHENG" of TCM or "DISEASE" of Western medicine must be taken into account before conducting TCM clinical trial because it relates to whether the results of evaluation are recognized or not.

Western medicines are based on the one drug one disease model i.e. the same medication is prescribed to all patients diagnosed with the same illness. In TCM, each patient is prescribed with an individual formulation, based on the evaluation the patient's health conditions and the environment.

Differentiating Syndromes in TCM and Orthodox Diagnosis in modern medicine are both methods of research and therapies, which are from two different angles to explore the development process of diseases in physiology, pathology, prevention and

treatment. For thousands of years, clinical practices are the most important part of Traditional Chinese Medicine. It has two major characteristics: empirical and individuality. On one hand, it shows the clinical efficacy of Chinese medicine objectively, but on the other hand it also shows that the clinical conclusions are obtained in the form of case report that is subjective and poor in reproducibility in certain degree. It is very hard to implement a unified standardized treatment. In fact, regarding to the recognition of ZHENG, so far people still remain relatively vague. Traditional Chinese medicine over-emphasizes the importance of "Treatment based upon syndrome differentiation," but seriously neglect of " Dialectical Disease and Treatment". Differentiating Syndromes embodies the overall characteristics of Chinese medicine, but lack of objective parameters. The uncertainty of ZHENG makes the clinical practice and efficacy of Chinese medicine cannot be evaluated stringently so that many people think that Treatment based on differentiating syndromes are too flexible to rely. (1). ZHENG of Chinese medicine is set up in the concept of dynamic holistic approach, and is based on *Yin* and *Yang* and *Five Elements* Theory reasoning tools for living "gasification structure" (2). The content of ZHENG is to study the balance of *yin* and *yang*, and the movement of *qi*, function of five organs, blood *qi* and body fluid in the metabolic process and so on. What Traditional Chinese medicine lack is modern medical understanding towards the fundament of disease. In general, "ZHENG" is lack of an important content that is deep recognition of disease. In the evaluation of the efficacy of Traditional Chinese medicine based on "disease" as standards is more easily accepted by international mainstream medicine.

2.2 Current Research directions taken by TCM practitioners

Thousands of years' clinical practice is the most important part of Traditional Chinese Medicine. There are two characteristics of TCM (empirical and individual), therefore it is difficult to perform standardization.

There are several different theories that build the basis of Traditional Chinese Medicine. This combination of basic philosophical theories is used to explain the interaction of all things in nature. The amalgamation of the theoretical systems developed into one of the oldest medical systems in the world. Allowing for expansion and interpretation, it gives the practitioners the freedom to think and develop organically. It is determined that the efficacy evidences of TCM were largely based on case reports with more imagination and less objectivity. Although TCM is effective historically, in the absence of convincing evidence, it tends to be excluded from the mainstream medicine, since the latter has developed faster and faster.

2.2.1 The philosophy of TCM

A very important philosophy in TCM is to cure disease before it has happened (治未病)—the prototype of preventive medicine. Important theories of TCM were based on the observation of nature or natural phenomena. They reflect the viewpoint of plain materialist dialectics, which maintain that the world is a material world in which all things are interrelated and interactive.

The theory of *yin* and *yang* is probably the single most important and distinctive theory in TCM. It could be said that all TCM physiology, pathology, and treatment can eventually be explained by the relationship between the forces of *yin* and *yang* (53, 64). All manifestations of nature are thought to be composed of a specific balance between these two forces. Health is also thought to result from an appropriate balance of *yin* and *yang* in the body, an imbalance of which may lead to a variety of diseases.

Some talents in China began to summarize those phenomena and sublimated to theory based on their philosophical and social knowledge at that time. The theory is the original TCM. Thus TCM handles human physiology and pathology following old Chinese philosophical thinking. In the following centuries, accumulation of experiences and addition of relative knowledge (such as clinical observation data and less anatomical experience) made TCM developed. The terminology of TCM is partially originated from Chinese philosophy. Other terms in TCM, even same as those in modern medicine, have completely different meanings. It is believed that to understand the physiology of TCM, to some extent, one should have some knowledge about Chinese philosophy (75).

Traditional Chinese Medicine is both an art and a science of healing, based on the harmonious coexistence of *yin* and *yang*. In spite of many scientific developments and innovations, TCM philosophy and principles are still deeply rooted in the *yin* and *yang* theory, handed down through many generations of theories and practices.

The fundamentals of TCM origination and development in theories are influenced by Chinese ancient philosophical thought, which is different from the western medicine. In western medicine affected by European conventional materialism philosophy, especially Atomic theory, element theory, was more emphasizing on structure restores, dissecting analysis and experimental comparisons (6). However, the core of philosophical thought of traditional Chinese medicine is *vital essence theory* (精氣學說), *Yin* and *Yang* theory and *five elements* theory.

Vital essence theory is considered a primitive monism of world, *Yin* and *Yang* theory is primitive dualism of world, which divides the world into two sides i.e. all object bisects and it is believed that any object has feminine and masculine aspects, mutually interacting and balancing.

Modern Western medical philosophy only began to emerge around the fifteenth century, although philosophies about science, nature and medicine did exist prior to this. In Western medicine, the body is viewed as a "machine." It has working parts that are replaced as they become worn out or break. One 17^{th} century physician even likened the body to a well-made clock. In the Western world, reality is "matter." It is solid and it can be touched. This view is established early in the training of Western medical health-care professionals and is exemplified in many common mechanistic analogies to human body parts. For example, the heart is described as a pump, the lungs are considered bellows and the nervous system is viewed as an elaborate telephone network.

In TCM, by comparison, everything is described in terms of nature. For example, the body is viewed as a garden, the health-care professional works as a "gardener" to maintain homeostasis of the garden, and their patients are considered to be an integral part of the Earth.

Eastern and western viewpoints clearly show that although doctors strive to cure the patient, they take very different routes. Western ideologies direct the doctors to isolate the disease, identify it, and then set procedures to eliminate it. This method has very often been likened to the car mechanic when servicing a car, the faulty part is found and either fixed or replaced. Even with the incorporation of new methodologies, the older ideas of viewing things in an isolated way are still the norm.

Traditional Chinese Medicine embodies rich dialectical thought, such as that of the holistic connections and the unity of *Yin* and *Yang*. It is this set of philosophies, which from the outside seem overly simplistic, that has allowed TCM to survive five thousand years, even with the emergence of western medicine.

2.2.2 Diagnosis in TCM

In traditional Chinese medicine, symptom-complexes do not describe diseases. They describe the functioning of the whole body at a definite time or stage of a disease. TCM practitioners make a diagnosis according to the "*eight guiding principles*" 八綱辨證 (*yin* 陰 and *yung* 陽, exterior 表 and interior 裏, cold 寒 and heat 熱, deficiency 虛 and excess 實), the state of *qi* and blood, the theory of the channel, the theory of the organs (*zung-fu*), the etiology of disease, and so on (7). The process starts with an assessment of cold and heat (i.e., retarded or accelerated metabolic activity) and continues with an assessment of a state of deficiency or excess (hypofunction or hyperfunction) of an organ or other factors. The process moves on to assess whether the condition is internal (affecting deeper layers of tissues and function) or external (affecting superficial layers). Finally, there is assessment of *yin* and *yang*. Accordingly, it is impossible to obtain exactly the same symptom-complex for any two patients, needless to mention a group of patients (8). Western medicine views a disease or syndrome as pathological changes of specific biological processes (9). As a result, the syndromes in Chinese medicine do not always correspond with Western classifications of diseases and syndromes. As symptom-complexes differ in different patients, the prescriptions of herbal medicines for treating them must differ too. Thus, it is nearly impossible for traditional Chinese medicine to conduct a stringent randomized clinical trial.

2.2.3 Treatment principle in TCM

Non-specific treatment (非特異性治療)

The therapeutic principles and goals of traditional Chinese medicine (TCM) are different from those in Western medicine. Most Chinese formulations contain a

mixture of herbs. Complicated ingredients in herbal formula and multiple targets in treatment mechanism have determined the characteristics of TCM as non-specific therapies. There are different methods of classifying the ways in which these can be combined. When combined, two biologically active substances can be observed to have the following effects: mutual accentuation, mutual enhancement, mutual counteraction, mutual suppression, mutual antagonism, and mutual incompatibility. The principal ingredient is a substance that provides the main therapeutic effect; the second principal ingredient enhances or assists the therapeutic actions of the first. The rest serve one of the following functions: treat accompanying symptoms, moderate the harshness or toxicity of the primary ones, guide the medicine to the proper organs, or exert a harmonizing effect. TCM aims to correct maladjustments and restore the self-regulatory ability of the body, and not to antagonize specific pathogenetic targets. TCM does not focus solely on the disease defined by specific pathological changes, but instead concentrates on the overall functional state of the patient. Physiology in TCM is featured with self-controlled system discrimination and its pathology is featured with dynamic changes in the system (whether direct or indirect, specific or non-specific). The therapeutic mechanism in TCM focuses on enhancing human body's resistance to diseases and prevention by improving the inter-connections among self-controlled systems.

Individualized treatment (個體化治療)

Treatment based on Differential Syndromes of Traditional Chinese medicine is a typical "individual treatment". During the process of diagnosis and treatment, Traditional Chinese medicine focuses on the analysis of personality characteristics. Treatment based on Differential Syndromes initially came from the "*Treatise on Febrile Diseases*"《傷寒論》. Treatment based on Differential Syndromes is the unique

characteristic of TCM; its merit is individualized treatment, the right medicine for different individuals. However, the efficacy evaluation to satisfy the requirement of repeatability is very difficult. It is also difficult to apply the method of evidence-based medicine in TCM research. That is why the evidence of TCM usually comes from case reports, which is not the strong evidence and not accepted by mainstream medicine (1210). However, as an emerging medical science, evidence-based medicine is not omnipotent. For individualized treatment promoted by TCM theory that treatment is based upon syndrome differentiation, it is worth to explore if randomized controlled clinical trial is applicable in TCM clinical trial. How to deal with "*treating same disease with different methods* 同病異治" and "*treating different diseases with same method* 異病同治", and large variability in interventions of TCM should be methodologically explored.

The origin of Traditional Chinese medicine is the result of clinical practice. A long history of several thousand years is the process accumulating experiences, understanding, practicing and re-recognition, and it formed its unique theoretical basis and methods in health care and disease prevention. Traditional efficacy evaluation of TCM was mainly based on the TCM practitioners' experiences and the individual patients' subjective feelings. These experiences are challenged in their poor repeatability or even unrepeatable when they are promoted in clinical practice. How to prove the efficacy of TCM and apply it widely in clinical practice requires verification by use of modern scientific research methods in clinical trial design and conducting.

2.3 Current research status in Chinese medicine

Currently, the status of TCM researches is in its bottleneck.

The reasons for the lack of progress can be summarized as:

- Clinical efficacy of traditional Chinese medicine were fewer demonstrated by RCT
- Compared with single compound, the advantages and efficacy of herbal mixture have not yet been established. This brings another question: whether or not Traditional Chinese medicine therapy will eventually be replaced by the treatment of single compound of the mainstream medicine?
- Quality control of TCM is very difficult to maintain because the herbs are grown wildly, and the composition of herbs are variable according to the changes of maturation, growth status, and cultivation condition.
- It's very hard to understand well the structure of herbal medicine because the effective compounds have more than one function, which may involve a variety of cells and tissues.
- The concept that Traditional Chinese medicine is safe, is due to they are "natural" without artificial element, and have been used for several thousands of years. However, this concept ignores the evidence of the toxicity of herbal medicine itself.
- Chinese medicine treatment in the point of view of methodology is quite specific and symbolic and this has not been approved by the current technology. Furthermore, the terminology of Chinese medicine is indeed quite different from those of the mainstream of medicine, which hinder the practice and the application of advanced methods to study Chinese medicine.
- Restoring methodology of science and technology is not sufficient to master the inherent complexity of plant chemicals and the diversity of medicinal herbs.

Recently, Chinese herbal medicines are being studied worldwide (11). An increasing number of researchers are focusing their attention on developing drugs from

traditional Chinese medicinal herbs and identifying the active ingredients of these herbs and their pharmacological mechanism of actions (12).

New scientific technologies, including tissue culture and biological screening methods, continue to improve the research process. For multi-component herbal prescriptions, standardization and quality control, including GAP (Good Agricultural Practice), GMP (Good Manufacturing Practice) and GCP (Good Clinical Practice), are performed to guarantee high quality and consistency. Pharmacological, and drug administration, distribution, metabolism, excretion, and toxicological (ADMET) studies are used to validate the quality, efficacy and safety of herbal preparations.

2.3.1 Authentication--Identification of starting raw materials

Authentication and quality consistency are the basic requirements for herbal medication. To insure chemical uniformity, it is necessary that the starting plant material for manufacture should be accurately identified and authenticated by their scientific names (Latin binomial) in the form of a voucher specimen. The need for the scientific name is important because a common name is inadequate as it often refers to more than one species. Such as in the case of *Astragalus*, *Huangqi* is considered the common name for *Radix Astragali*, *Astragalus membranaceus*. Even when plants do not have the same common name, problems still arise when common names are similar. This can lead to accidental substitution of a safe species by a toxic species.

Plant species need to be identified properly. Within each species, there is considerable variability in the content of specific active compounds, depending on such variables as soil conditions, temperature, precipitation, and time of harvest. There is also limited understanding of the differences between medicinal plants grown in the wild, and those cultivated commercially. For safety, plants used in TCM should be free of heavy metals, chemical, pesticides and microbial contaminants.

For example in Danggui Buxue Tang (DBT) study, which is one of the simplest ancient herbal formulae. The formula consists of only two herbs: *Radix Astragali* (RA, Huangqi) and *Radix Angelicae Sinensis* (RAS, *Danggui*) in a weight ratio of 5:1.

Some Chinese medicinal materials with excellent quality are only produced in certain regions of China, which are often referred to as 'the best growth region' or '*Daodi*'. Therefore, how to authenticate and choose the best RA and RAS plays a critical role in ensuring the quality of DBT (**Figure 2-1**). In DBT authentication study, amounts of total saponin, total isoflavonoid and total polysaccharides were determined in RA collected from various regions (**Figure 2-1 a**) and amounts of ferulic acid and ligustilide were determined in RAS collected from various regions and countries (**Figure 2-1 b**). The authentication studies showed that RAS from Gansu, China and RA from Shanxi, China should be used for DBT preparation (**Figure 2-1 c**) (13).

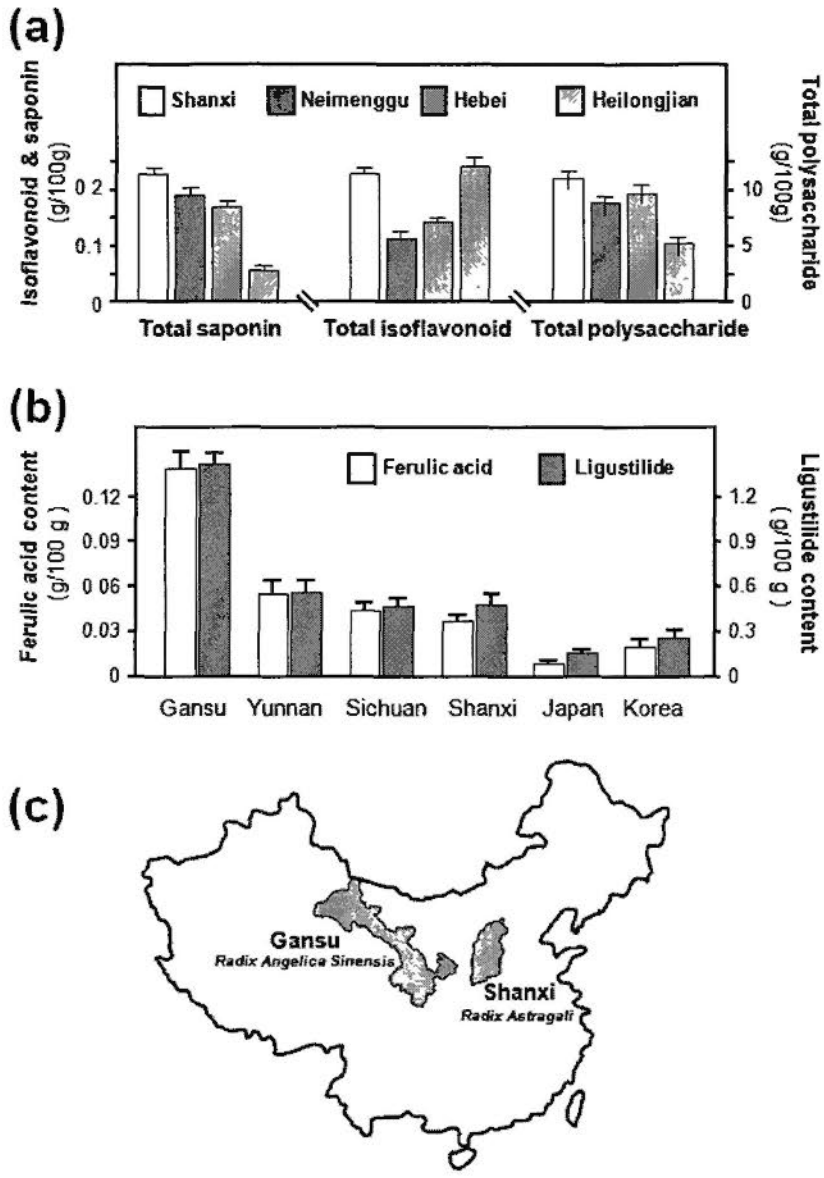


Figure 2-1 Determination of the active constituents in RA and RAS (13)

2.3.2 Proper preparation of herbal medication (GMP)

Quality of herbal preparation is very important as it may affect the outcomes of clinical trials and will determine whether or not the herbal preparation will be selected for further development (15). Guidelines have been established with respect to Good Manufacturing Practice (GMP) of investigational agents (16).

Reproducible efficacy and safety of herbal drugs is based on reproducible quality.

Authenticated raw material is the basic starting point for the development of a botanical product. However, harvesting, storing, processing and formulating methods may dramatically affect the quality and consistency of the final product by altering the desired marker components or by increasing the possibility of unwanted contaminants. Thus, validated methods to ensure quality control in manufacturing and storage are required tools for optimal efficacy and safety of the products. These controls are also critical for the evaluation of pharmacological, toxicological and clinical studies of the botanical supplements.

The key challenges in the development of analytical methods for botanicals and herbal preparations are: (1) analysis of marker or active compounds in a complex and sometimes unknown environment, (2) target analyses may be polar and thermally labile, (3) lack of chemical reference substances and certified reference materials, (4) selection of extraction method and (5) batch-to-batch variation of the composition of the plant materials obtained.

For the better quality of the finished product, the right sequence of mixture, the mixing time (30 min), the interim storage (2 weeks) and the filtration (sterile filter) are essential for avoiding unwanted reactions between the different extracts (for example major precipitations) and for ensuring stability.

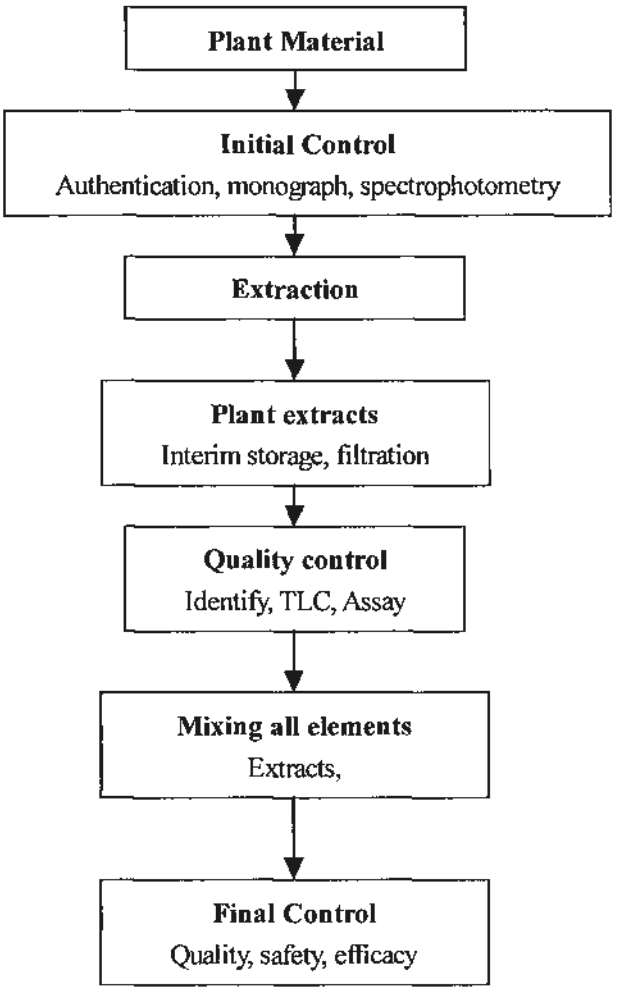


Figure 2-2 From herbal drugs to a multi-component herbal medicinal product: manufacturing and quality control scheme

2.3.3 Chemical standardization (Herbal drug standardization)

"Standardization is a system to ensure that every packet of medicine that is being sold has the correct amount and will induce its therapeutic effect" (17). Quality control standards of various medicinal plants used in healthcare system are becoming more relevant today in view of commercialization of formulations based on medicinal herbs. Because of varied geographical location where these plants grow, coupled with the problem of different vernacular names these plants are known by, a great deal of

adulteration or substitution is encountered in the commercial markets. Therefore, reproducible standards of each plant are necessary for effective quality control. Due to the natural heterogeneity, the quality of herbal starting materials obtained from wild collections shows more and more fluctuations. Thus cultivation of the most important medicinal plants has been considerably promoted. Standardization serves a number of purposes: 1) batch to batch consistency, 2) confirmation of correct amount of extract per dosage unit, 3) positive control to indicate possible loss or degradation during manufacturing.

Each of the principal herbs is standardized as to the content of the major active compounds (many of which might be unknown). The objective is to establish a chemical "fingerprint" that meets certain standards for each lot of a particular herb. The actual formulation always will be based on a mixture of such standardized herbs.

2.3.4 Adoption of modern methodology in pharmaceuticals

An increasing number of researchers are focusing their attention on developing drugs from traditional Chinese medicinal herbs and identifying the active ingredients of these herbs and their pharmacological mechanism of actions (12, 18). Recently, it has been suggested that herbal remedies need quality control and rigorous clinical trials (19, 20). However, the real efficacy and toxicity of TCM would not be tested adequately using the modern evaluation methodology for efficacy evaluation.

Chinese herbal medicines are complex mixtures, containing usually hundreds of chemically different constituents but only a few compounds are responsible for the beneficial and/or hazardous effects (21). Biologically active compounds form just a minute part of herbs being diluted with a large amount of proteins, sugars or tannins, which, in some cases, does not contribute to the pharmaceutical effect but they make the quality control of crude drugs. Therefore, very efficient and selective methods,

including the extraction techniques are required for identification and quantitative analysis of the active compounds or drug standardization. Chromatography and electromigration methods represent main techniques applied in this field due to their powerful separation efficiency combined with sensitive detection (2522).

Modern technologies being used or under development for the QC of CM include: microscopic analyses (morphological investigation and powder methods), chromatographic methods for fingerprint profiling [thin-layer chromatography, high-performance liquid chromatography, liquid chromatography and gas chromatography (TLC, HPLC, LC, GC), GC- and LC-mass spectrometry (GC-MS and LCMS), LC/MS/MS and capillary electrophoresis (CE)], DNA polymorphism methods to analyse DNA sequences and establish relationships between genomic DNA fragment patterns and ratios of active components, and biological methods or bioassays.

2.3.5 Pre-clinical study

Pre-clinical studies typically include toxicology and pharmacodynamics studies, they are essential components of drug evaluation.

Toxicological studies constitute an essential part of the effort in developing an herbal medicine into a drug product.

If the initial trial shows promising results, chronic and other toxicity studies in animals become important in that they would provide information on potential target organs of toxicity and a complete toxicity profile of the herbal medicine. If there existed previous human experience or animal toxicity studies that could support a longer term clinical trial, the chronic animal toxicity studies may be waived depending on the availability and quality of the existing data.

Toxicology studies of TCM include *in vitro* and *in vivo* tests. In vivo studies are

divided into animal toxicology tests and human safety studies. Animal experiments are mainly testing the toxicity of traditional Chinese medicine, including acute toxicity, subacute toxicity, chronic toxicity, reproductive toxicity, Genotoxicity, immune toxicity tests, and carcinogenicity

Based on clinical research necessity, toxicology research has the following main purposes: (14):

1) general toxicity
2) target organs or systems of toxicity
3) teratogenic, carcinogenic, or mutagenic potential of any botanical ingredient in the product
4) relationship of dosage and duration to toxic responses
5) pharmacological activity

However, the current safety studies for single herb or combination herbal formula of traditional Chinese medicine are far from enough, and they mainly concentrated in the acute toxicity and long-term toxicity studies; there are more recognition the toxicity of traditional Chinese medicine from clinical practice, less understanding of its nature and the mechanism in depth; more understanding of the acute toxicity that can be found on short-term and less understanding of their hidden or potential toxicity or organic toxic injury after long-term use. Over time, the concept of traditional Chinese medicine "non-toxic" is gradually formed. After thousands of years the natural environment and human living environment are changed. Most of the Chinese herbal medicine have been changed from the wild status to artificial cultivation. In the production of traditional Chinese medicine, old preparation methods have improved by using scientific methods of extraction and concentration. Some trace elements in traditional preparation significantly increase after adopting scientific extraction technology. Active ingredients significantly increase but at the same time the toxic

elements also increase. The commonly used herb *Danshen* for promoting blood circulation and removing blood stasis and classified as superior herb in S*hengnong Bencao Ji* was reported to have the action of promoting the transfer and proliferation of cancer cells in cancer patients (23). Other commonly used herbs such as *Acorus calamus Linn* Chang Pu (24), *Flos Farfarae* Kuan Dong Hua (25), were also reported having teratogenicity effects. So it is imperative to re-evaluate toxicity of traditional Chinese medicine.

2.3.6 Clinical study

Ancient efficacy evidences usually came from case reports that recorded in numerous ancient classic works (documents). Case report provides detailed information on individual patient that may be representative of a new condition and／or treatment approach. When several patients are involved this is considered as a case series. It is difficult to generalize results to other patients based on such limited numbers of observations. That is why we need a randomized controlled trial (RCT) with reasonable sample size to re-evaluate the efficacy of TCM therapy.

The use of RCTs to evaluate TCM is associated with some unique challenges. These include: design issues (e.g. identification of clinically relevant research questions, standardization of interventions, blinding issues, selection of control groups and placebos), recruitment issues (including randomization issues), and systemic issues (e.g. qualifications of researchers, difficulties obtaining approval from ethic committee, and funding issues). Nowadays most of the clinical trials in TCM are case reports or clinical observations, well-designed clinical trials following the principles of RCT are still fewer.

Even though Traditional Chinese Medicine are widely used in China and other countries, and because of its theoretical system and treatment principles can not be interpreted by using the pathophysiological mechanism of Western medicine, it has not yet been generally recognized by the modern medicine. The main obstacles include: 1) It is far from wide and depth in basic researches of active ingredients in Chinese medicine especially for the combination of herbs, and can not provide the main scientific evidences in ingredient, physical and chemical characteristics, biological activity and target; 2) Traditional Chinese Medicine emphasizes individual treatment according to differentiating syndrome; it is very difficult to achieve standardization for Chinese medicine that is limited in the scope application.

From the perspective of Western medicine, comprehensive studies of a medication including the active ingredient, mode of action, metabolism, are essential before it can be approved for human use. It is generally accepted by the US Food and Drug Administration (FDA) that TCM herbs that have been in long-term use is safe. Nevertheless, they need to undergo extensive tests to demonstrate efficacy against the particular disease. Such tests are time consuming and expensive.

Designs for clinical assessment of herbal medication may include: 1) randomized controlled trials (RCT), 2) clinical observation, 3) cohort studies, 4) case-control studies, 5) series cases reports, and 6) single case reports. Although methods such as blindness and placebo control may not always be suitable, we still need to emphasize the importance of applying RCT to clinical research on herbal medicine because they provide the best evidence (26) that is accepted by mainstream medicine.

Experts and physicians of Traditional Chinese medicine largely rely on case reports obtained from clinical practices without rigorous design. Its disadvantage is lack of bias control, and the results are usually unrepeatable.

At present, in the study of traditional Chinese medicine, case reports are still used by

many researchers. This is the basic TCM research method having been used for 5000 years. All TCM ancient classics came from the accumulation of case reports. Case report method objectively recorded the status of the patient after the drug administration; it still has its value even now. For certain rare diseases, the case report method is still worthy to use.

Before 1960s', because of the impact of ancient medical text and modern medicine, clinical efficacy evaluation in modern Chinese medicine is mainly embodied in the case reports and case summaries. Real clinical researches for Traditional Chinese medicine were started about 20 years ago and shown to be an increasing trend in recent years. However, a number of problems still exist in clinical studies (27, 28): poor quality of study design and report; improper application of randomization methods; insufficient sample size; unclear study parameters; difficult to standardize and quantify the efficacy parameters; the efficacy of the study unrepeatable; Efficacy indicators are mostly "soft" parameters such as clinical symptoms, lack of endpoint "hard" parameter (such as mortality) obtained through long-term follow-up. These issues affect the reliability of the study results.

At present, clinical trials usually use positive control, mainly due to 1) easy to pass the authority assessment: compared with positive drug, even though no significant difference the study drug could also be considered effective); 2) from the ethics point of view, placebo treatment may delay treatment, which is not in conformity with ethical principles.

2.4 Drug development—Phyto-drug to chemical drug

From the beginning of the 21st century, the research and development of plant medication are becoming prosperous. This is due to screening new drugs from

chemical compounds are more and more difficult. In addition, chemical drugs usually have insurmountable toxic effects and unwanted side effects such as drug-induced diseases, all these negative factors push people to look towards natural medicine. In natural medicine, the largest share is the plant medicine, which has become the focus of creation of new drugs.

Western pharmaceutical industries tend to approach TCM from a non-holistic perspective, searching for single bioactive compounds. The idea is to isolate and characterize those and use them as a template for drug lead optimization studies. This has been done over many years with great success, as an important part of drugs are derived from constituents from nature and not from the creation of chemical diversity within the constraints of the laboratory. Successful examples are *Qinghao* and anti-cancer drug. However, this approach for bioactivity screening removes the important basis of multiple component intervention, inducing synergy being the basis of TCM's holistic approach. This leads to multi-target of multi-dimensional pharmacology based discoveries and strategies. Synergy is an aspect that will be lost in a target driven single lead discovery programme with TCM.

New plant-derived medicines can come from three sources: single active principles, active fractions, and validated prescriptions.

In recent years, the health care paradigm has shifted from a focus on diseases to a holistic approach to health care. Medicines are needed not only for treatment of diseases but also for prevention of diseases and promotion of quality of life of an individual. The treatment of patients is based on a more holistic and integrated approach instead of "one-drug-for-one-disease" approach.

A pure chemical compound as a drug to treat complex modern diseases is very difficult. The systems biology approach, which contains a variety of chemical composition, can compensate for the inadequacy of modern medicine. The holistic concept of Chinese medicine might be an embryonic form of "holistic medicine". Individualized diagnosis and treatment of Traditional Chinese Medicine have many features of today's mainstream medicine towards personalized medicine. Chinese herbal formula is usually formed by a number of Chinese herbs, containing a variety of chemical composition, with the features of multi-targets action mode. All these features are in line with modern mainstream medical development.

Clinical trials for chemical drug demand the details of the chemistry, the mode of action and metabolic pathways before clinical trials are implemented. In the past 60 years, a lot of studies for the herbal medications have been done on the basic understanding and yet not much have come out. Until October 31, 2006, the US Food and Drug Administration (FDA) approved the only one new drug application (NDA) for marketing of Veregen (sinecatechins) from botanical Green Tea, for topical treatment for perianal and genital condyloma (29). Even a single herb contains so many complicated ingredients that many years of research might not yield much fruit.

In Western countries, the plant drug study can be divided into three levels; 1) Extracting the active ingredients from the plant, which is equivalent to Class I new drug in China; 2) Extracting the active fractions from the plant, which is equivalent to Class II new drug in China; 3) Single or multi-plant-based preparation, which is equivalent to Class III new drug in China.

In China, basically there are two types of orientation in research and development of plant effective parts: 1) extracting effective fractions from single herb; 2) extracting effective fractions from herbal formula. The latter is more in line with the theory of

Traditional Chinese Medicine. For multi-component herbal prescriptions, standardization and quality control, including GAP (Good Agricultural Practice), GMP (Good Manufacturing Practice) and GCP (Good Clinical Practice), must be performed to guarantee high quality and consistency.

Conventionally, for single active compounds, its discovery and drug development involve highly efficient bioactivity-directed fractionation and isolation (BDFI) coupled with structural characterization, analog synthesis, and mechanism of action studies. There is interesting worldwide research in new drug development from active ingredients of plant, the more prominent examples being artemisinin, vincristine and paclitaxel.

Artemisinin having a high degree of specificity of anti-malarial effect is the active ingredient extracted from the Chinese plant Artemisia annua. Initially, the anti-malarial effect was found in the Chinese Herbal Formula named *Qinghao Biejia Tang* (青蒿鳖甲汤), after pharmacological screening, artesunate was found to be the essential herb with anti-malarial effect in the formula, and then from the herb Artemisinin was isolated. It is a rare single-target herbal preparation.

Vincristine is an efficient and effective anti-leukemia component, which was extracted from India folk anti-malarial herb Catharanthus roseus. The initial studies found that there were more than 60 kinds of Vinca alkaloid, among them vinblastine has better anti-leukemia activity. Finally Vincristine was obtained, which is nearly 7 times in anti-leukemia activity and with less toxicity, however its content is only about five millionths, and is difficult to be taken for industrial production. At the end, vincristine can be achieved for industrial production by low-temperature oxidation of vinblastine.

Taxol is an anti-cancer active ingredient extracted from the bark of a unique tall yew tree in China. It has good therapeutic effect for breast cancer and other kinds of cancer,

so that it is quite popular in the global pharmaceutical market. Some researchers tried to synthesize taxol by using chemical synthesis method but it failed because of its complex molecular structure.

The above-mentioned successful examples give very good idea in modernization of traditional Chinese herbal medicine. Extracting effective fractions from Chinese herbal formula should be the main purposes of modernization of Traditional Chinese Medicine, because the Chinese herbal compound is the essence of TCM. This also accords with our advocating principle—efficacy-driven approach. If we have no need to blindly seek extracting active ingredient from single herb and purifying it and then artificially synthesizing it, and eventually develop it into a chemical drug. The past 60 years' experience has proved that this drug development procedure is a very hard road. Investing huge resources may not be able to achieve the desired objectives. Therefore, it is necessary to explore another way by adopting the principle of efficacy-driven approach, to study the efficacy of classic herbal applications. The following chapters will discuss this issue in detail.

2.5 Evidence-based TCM research

Attitudes towards TCM have changed in spite of that solid scientific evidence is still required. It has been realized that the effects of TCM have been "field-tested" by tens of thousands of people for hundreds of years (30). In fact, learning from the history can give people more confidence in terms of "evidence of practice" than any single methodologically rigorous clinical trial, and it is the "evidence of practice" that gives birth to the holism philosophically than scientifically. The knowledge that is scientifically correct is not always useful to the practical therapies of TCM, and thus careful attentions must be put on its holistic medical pattern.

As a complex system, TCM is usually used in combination of several herbs with multi-target and multi-level actions involving a number of genes and cells for regulating the body's overall balance.

Modern medicine in study of TCM usually disassembles the herbal formula into individual herb and looks for the active ingredients of the herbs. However, these research methods usually ignored the synergistic effects of TCM herbal combination principles. Therefore, in order to maintain its strong vitality, TCM should not confine itself to the "holistic concept" and refuse further development by only remaining at the level of extraction and purifying active ingredients. TCM should make use of modern advanced biomedical research methods to enhance its research level. To achieve that, a lot of things should be learned from modern scientific research methodology, and apply them in TCM research.

Treatment based on differentiating Symptoms of Traditional Chinese medicine is a kind of original evidence-based method, but the "evidence" in Traditional Chinese medicine and evidence-based medicine are quite different. Modern evidence-based medicine comes from well designed randomized clinical trials, however, evidences of TCM were usually obtained from empirical or experiences which are not accepted by mainstream medicine (31).

Although methods such as blindness and placebo may not always be suitable, the mainstream medicine still emphasizes the importance of applying randomized controlled trials to clinical research on herbal preparations because they provide the best evidence that the study results come from planned intervention.

With the rapid development of science and technology, how to draw and apply the

theory and methods of modern science and technology (including Western Medicine) to promote the development of Chinese medicine is a challenging issue. Recalling the history of natural science development, it is inseparable for the development of any subject that is always associated with the methodology breakthrough and innovation. Since the 80's of last century, clinical epidemiology and evidence-based medicine (EBM) have been recognized by medical profession as an extremely important methodology in guiding clinical practice, planning and clinical decision-making (32, 33, 34, 35). There are many major differences between Chinese medicine and Western medicine in understanding the human body, law of life, and clinical thinking and practice. Some of them are advantages of TCM, however some are limitations of TCM that were brought about by historical reasons.

The concept of evidence-based medicine (EBM) has been widely adopted by orthodox medicine. Proponents of EBM have argued that herbal medicine ought to be subjected to rigorous, controlled clinical trials in order to assess their efficacy.

The recent push to apply the principles of evidence-based medicine to TCM is important. Evidence of efficacy and safety of herbal medicines, like all forms of medicaments, can best be generated by clinical studies under Good Clinical Practices (GCP). Unfortunately, very few such studies on herbal products have been published. In the past two decades, most clinical studies have been conducted in Europe on single herb preparations, with very few being concerned with herbal mixtures such as those commonly found in TCM.

The highest-quality scientific evidence, according to the hierarchies of EBM, derives from large, randomized, controlled clinical trials, with lower-quality evidence from less rigorous study designs (36).

Knowledge in medicine can come not only from clinical trials but also from simple clinical observation, and this does not appear to be in doubt. Empirical evidence

comes in many forms, from the results of large clinical trials to the simple observation of individual patients. But knowledge from divergent sources may appear to conflict. EBM gives general preference to knowledge obtained from controlled clinical trials; this preference, however, may not be warranted (37).

Although there are many obstacles of TCM clinical studies in methodology, it is possible to conduct a rigorous designed clinical trial according to the principles of randomized controlled clinical trial. If a clinical trial is designed and implemented properly, clinical efficacy of TCM treatment could be proved. However, if the design is unreasonable, and the implementation is improper, even if the Chinese medicine is effective, the opposite conclusions may probably result. Therefore, the method of evidence-based medicine is a double-edged sword, it can verify the effectiveness and science of Chinese medicine, but if used improperly, it may fundamentally negate Traditional Chinese Medicine.

In general, research methodology of traditional Chinese medicine focuses on macro-level, holistic and intuitive approaches, therefore more macro description and less precise quantification; more general reasoning and less concrete analysis, more intuitive observation, and less experimental research are done and yielded corresponding results. These in certain degree hinder the development of Chinese medicine.

So far, randomized controlled clinical trial is still recognized by the medical profession as a "golden standard" method in evaluation of effectiveness of an intervention. However, due to its limited scope of application and obstacles in methodology, as well as the special characteristics of TCM in therapeutic treatment, many TCM therapies are not evaluated by these recognized methods, which prevented acceptance of TCM by mainstream medicine.

Chapter 3

Modern medicine and Adjuvant Therapy of Traditional Chinese Medicine

Although the history of modern medicine is much shorter than Traditional Chinese Medicine, its development speed is very fast and it has played critical role in maintaining human health.

While modern medicine has developed a very well established system of clinical research which insists on evidence based methodology relying heavily on biostatics, traditional medicine has not developed its own system of research. Since there are many problematic areas in modern medicine, which are lack of solution, and traditional medicine possessed advantages in those areas; it is necessary that experts in both areas should work together. One way is to stick to requirements of modern clinical trials as much as possible. Obvious obstacles include the common lack of uniformity: among the supply of herbs, and consistency of their quality. Another way is to apply the Efficacy driven approach which implies the following:

i) Getting a simple herbal formula to try solving one difficult clinical problem and start an evidence-based clinical trial using methodology acceptable to standard clinical trials i.e. randomized, placebo-controlled;

ii) Organizing parallel laboratory experiments to understand the mode of action;

iii) Making sure that the quality of herbs or their extracts are of the best standard and

iv) Once proven efficacious in the clinical trial, optimization of the formula will give an up-graded product.

The components of Chinese herbal medicine are extremely complex, the action mode has not been well understood, and the safety evaluation is need to improve. Therefore, in evaluating the safety, efficacy of Chinese herbal medicine, a comprehensive approaches are required. Simple or one aspect research method cannot fully reflect the safety and efficacy of Chinese herbal medicine, and finally may lead to wrong

conclusions.

3.1 Success of modern medicine

In the 5th century BC, an ancient Greek by the name of *Hippocrates*, made an impressive career as a healer and is now known in the West as the father of medicine. He put forward a very detailed concept of healing that was very similar to the theory of ancient Chinese medicine. The 5th century BC was the time of "*Spring and Autumn Period*" in China. "*Huang Di Nei Jing*" was believed to be written during this period (1-4). There were a lot of common concepts in the Chinese medicine and Western medicine: importantly both emphasized the macroscopic, holistic approach and both pursued a physiological balance or homeostasis in the treatment of diseases (5).

After 200 AD, two medical masters were born about the same period in the West and East. *Galen* in the West, and *Zhang Zhong-jing* in the East were equally important. *Galen* founded the positivism of modern medicine. He is called the originator of positivism of modern medicine, or the founder of Western medicine. *Zhang Zhong Jing* in China wrote "*Shanghan Lun*" (傷寒論) and "*The Synopsis of the Golden Cabinet*" (金匱要略) which laid the foundation of *treatment based on syndrome differentiation* (辨證論治). From this time, traditional Chinese medicine and Western medicine began to be differing. Western doctors after *Galen* wanted to understand the structure of the human body. However in China, started from *Zhang Zhong Jing*, the theory of *treatment based on syndrome differentiation* (辨證論治) was established: which meant no matter what was really happening, treatment was planned according to symptoms. Since the establishment of the theory of positivism, *Galen* explored the anatomy of the human body. In China, based on *Zhang Zhong Jing's concept of syndrome differentiation*, the therapeutic varieties entered a rapidly developing stage. The "thousand years of darkness" in the West obstructed the development of medicine which remained stagnant. The foundation of medicine laid by *Galen* almost collapsed. However in China, Chinese medicine flourished on fertile soil of the late *Han* and

prosperous *Tang Dynasty*. In the *Yuan Dynasty*, *Wei Yi-lin* (危依林) innovated a suspension method in the treatment of fracture《世醫得效方》, which was 600 years earlier than a British doctor Davis who put forward the apparently similar Stimson method in 1924. It was a major breakthrough in medicine science (6). Chinese medicine was developing very fast during this period.

However, after the Renaissance in the west the situation changed. Copernican heliocentric theory was proposed. At the same time, Andreas Vesalius who is often referred to as the founder of modern human anatomy wrote an influential book "*On the Structure of the Human Body* ". This book and Copernicus's heliocentric greatly shocked the science world in the west and promoted the development of sciences. From 1543 onwards, Western medicine kept its rapid developmental pase for three to four hundred years. Histology, embryology, microbiology, bacteriology, and cytology were thoroughly studied. The structure of the human body and its function was more and more understood. From this time, the differences between Chinese medicine and Western medicine became crucial. When Western medicine made continuous progresses, Chinese medicine was almost stagnant (7).

While Western medicine has made significant progress in the past century, recent 20 years it made the most spectacular advances. The achievements were the results of a number of factors, which included:

- The persistence of a scientific spirit which kept exploring unknown phenomena of the world that we lived in
- Quest for precision which could be acquired via repeated investigation and tests
- Pragmatic objective approaches gave outstanding results
- Advances of technology and availability of quality tools for experimental application

Since the beginning of last century when Western Medicine entered into China, it has developed rapidly and was apparently replacing Traditional Chinese medicine.

Western Medicine, also called Modern Medicine, is based on an approach that insists on reproducible observations which followed some theory or hypothesis, to be validated with tests and experiments. It has built up, from bedside observation, then microscopic observation, biochemical, and other analyses made available by the continuous development of scientific methods. Since the period of Enlightenment in the eighteenth century in Europe, bedside medicine developed, and grew into laboratory medicine. Its array of technology for diagnosis and therapy has given much success in the combat against diseases. Tremendous achievements have been made in modern medicine in the past that provides fast relief of symptoms for specific disease.

Modern drugs in the form of single-chemical entities have been successful in the treatment of acute conditions such as infectious diseases. Drug action is fast and predictable, leading to a favorable resolution of a critical problem in a short period of time. The historical paradigm "one drug, one target" has resulted in the identification of many effective chemical molecules that affect specific proteins.

The major successes of modern medicine occurred all within the last century. All advances were based on a thorough successful exploration of the basic medical sciences.

Traditional Chinese medicine dramatically differs from modern scientific medicine in its basic medical orientation, physiological theories, etiology, diagnostics, therapeutics, and pharmacology. For instance, while modem scientific medicine views the essence of illness as anatomicopathological, traditional Chinese medicine views it as symptom-complex (*zheng* 証) of the whole body. While scientific medicine identifies the sources of illness as disease entities, Chinese medicine identifies them as imbalanced climate and/or emotional factors. While scientific medicine uses advanced lab and mechanical investigations as diagnostic means, Chinese medicine uses sensory observations (looking, smelling, asking, and feeling) to locate problems. While scientific medicine emphasizes pathological anatomy, Chinese medicine

focuses on the patient's complaint and actual experience of being sick. While scientific medicine aims at curing diseases, Chinese medicine appeals to balancing functional factors. While scientific medicine employs chemical drugs or surgeries, Chinese medicine appeals to natural herbs or simple needles. From above-mentioned we can see the big difference between modern medicine and Traditional Chinese Medicine.

Success in modern clinical medicine, according to proponents of EBM, depends not upon the production and explication of the most coherent or convincing theory of disease, but upon the completion and utilization of systematic studies designed to demonstrate the effectiveness of particular treatments for particular conditions (8).

3.2 Limitation of modern medicine

It is undeniable that modern medicine has made great progress in the past century, especially in the last decades. Modern medicine has become a very complete and sophisticated special subject, with not only a complete theoretical system, but it has also developed special techniques for treatment purposes, such as test-tube babies, organ transplants, etc. However, there are still many inadequacies in modern medicine (**Table 3-1**). It is obviously not the final solution for all human health problems (9). For incidence, modern medicine is still offering no effective methods to treat a lot of virus infections, cancers, endocrine disorders, autoimmune diseases, degenerative diseases, chronic diseases, functional disorders, and age-related disease.

Table 3-1 Special areas in modern medicine that could benefit from alternative medicine (10)

1	Allergic conditions
2	Autoimmune diseases
3	Cancers
4	Chronic pain
5	Chronic derangements

6	Degenerative diseases
7	Nerve damage
8	Viral infections
9	Other areas where modern conventional therapy fails

The following factors also influence the development of modern medicine:

- Modern medical aims at single-target actions using simple chemicals, complex diseases then may not benefit
- Single chemical entities might deal with anomalies in the target cells, tissues, or organs effectively yet cause a loss of homeostatic balance manifested as side effects
- Chemical drugs often develop tolerance or resistance after long-term use
- Research and development of therapeutic agents is expensive
- Complex pathology might need a combination of agents that aim at multiple targets

Most diseases are the result of multi-factorial pathological changes at tissue, cellular and even molecular-levels. A single chemical composition used as a drug to treat modern complex diseases is therefore not comprehensive. Therefore, for the complex chronic diseases, modern medicine has limited offer, and the effects are far from being satisfactory.

Traditional Chinese medicine has accumulated rich clinical experiences. It is taking a "holistic approach". While at the same time, individualized diagnosis and treatment are considered necessary. The herbal medicines used are usually composed of many herbs, which contain a large variety of chemical compositions which must be targeting at multiple sites. All these features of TCM are similar in direction with the mainstream of modern medical research (11).

Rapid development of modern science and technology and multi-disciplinary

researches show that the recognition of diseases has changed. The recognition of diseases is not limited to the biomedical model, but has developed into a biological-psychological-social medical model. The causes of diseases are better revealed as more and more complex. For a specific disease, the causes could be multiple. Using one chemical with its defined target in treatment of diseases, not only may there be multiple side effects, but without a holistic approach, it may also be ineffective. For acute conditions, modern medicine has a lot to offer. For chronic or degenerative functional disorders, modern medicine is imperfect.

At present, development of new chemical drug is confronting difficulties due to the required expense and long duration needed. The number of new chemical entities being studied for new drug application is becoming less and less. Pharmaceuticals have been traditionally designed to target individual pathological changes in a disease system. In complex chronic diseases, an alternative way to clear-complex pathology may have to involve multiple targets (12).

3.3 Approaches of Chinese medicine

Modern medicine has its strengths and weaknesses; traditional Chinese medicine also has its strengths and weaknesses. How can the two systems complement each other is an important issue we are facing now.

With its multi-target effects, TCM is particularly suitable for the treatment of diseases with complex etiological causes such as cardiovascular disease, asthma, and other long-term illnesses (13).

The standardization of TCM depends on the authentication of the identity of Chinese medicinal materials. Therefore, the authentication and quality control have been for many years, considered to be one of the keys for TCM to enter the world market (14).

One of the characteristics of traditional Chinese medicine is related to holistic approach. It is soon found that the higher the purification, the less the efficacy becomes. Sometimes the isolated natural medicine components are not as active as

expected and can be even toxic (15). The chemical marker of a single herb is not always representative of its biological activity, hence its quality. New herbal preparations made with modern technology have been found to be less effective than the traditional decoction. In spite of the controversies, the material basis of TCM efficacy is still its chemical composition. After a herbal formula is formed, the active ingredients of the single herbs will reinforce to generate new effective substances to produce synergy or reduce effects through mutual antagonism, the result of which could be lowering of toxicity, or offering the side effects either positively or negatively. It is believed that because of the synergistic properties of TCM, it is hard to achieve the desired effects of the expected efficacy of a TCM herbal formula by using only the extracted active ingredients.

Using modern science and technology to study traditional Chinese medicine could be a practical way to modernize Chinese medicine.

Modern pharmacology is used to study the efficacy of Chinese herbal compound. Pharmacokinetics and pharmacodynamics are adopted to identify the mechanisms of action and metabolism of the herbal extract. Animal models are created to validate the *in vivo* efficacy. Toxicological tests are performed to evaluate the safety according to Good Laboratory Practice (GLP) standards, and to find out the safety dose. The efficacy and safety evidence derived from animal studies must be confirmed in clinical trials. Clinical trials should be implemented in accordance with Good Clinical Practice (GCP) standards otherwise the results are not accepted by mainstream medicine. Well-designed clinical research is crucial for the efficacy and safety evaluation of Chinese medicine. Improper designed clinical trial may not only fail to prove the potential efficacy of a Chinese herbal formula but may unnecessarily lead to condensation. When the pre-clinical and clinical research has proved its efficacy and safety, the next important step is to maintain the efficacy of new batches of the same herbal items. Manufacturing procedure according to Good Manufacturing Practice (GMP) standards would be the final step in the production of a new product.

Research activities in traditional Chinese medicine have, to date, focused on the

search for relevant active substances and mechanisms of action. This research approach is shaped partly by the conventional drug development model, which commences with determining the mechanism of disease, followed by the design and synthesis of therapeutically active compounds or molecules, animal and in vitro studies, and finally clinical trials in the humans. The past 60 years' research experiences have shown that the success rate of TCM in Research & Development (R&D) is very low if the development model is entirely following the chemical drug R&D model. Only a few examples of success could be identified which include the antimalarial drug artemisinin, and a complex of anti-cancer drug Vincristine that was isolated from botanicals and then chemically synthesized for mass production. The cost of chemical drug development is extremely high; it also needs a very long duration. Currently, chemical drug development is usually started from an understanding of action and then screened out desirable leading compounds from botanicals, assessed the safety then synthesize artificially the desired ones. Finally, clinical trials are conducted to evaluate its efficacy. The process of drug discovery for a pharmaceutical drug has been estimated to take an average period of 10 years at a cost of around 800 million US dollars (16). It is estimated that only one in 5000 lead compounds will successfully advance through clinical trials and be approved for medical use (17). Traditional Chinese medicine has a long history of extensive use. Randomized double-blind placebo-controlled clinical trials are used to prove its efficacy, and then to perform the further in-depth and targeted studies is a very effective way, which is commonly known as efficacy-driven approach. If a herbal therapy does not work clinically, it should be discarded and not subjected to further investigation. This efficacy-driven approach model avoids a large number of unnecessary basic researches, reducing the study cost, shorten the development cycle, and saving much funding.

In the efficacy-driven approach, investigation into the mechanisms and the search for active substances may follow after the clinical efficacy is firmly demonstrated (18). Scientific evidences of TCM effectiveness are of utmost importance. Scientific

evidence refers to quantifiable data in human clinical trials, epidemiological evidences, animal studies and evidence of biological activity from in vitro work. The greater consistency of evidence across all these kinds, the higher the strength of evidence. Almost without exception, all claims based on scientific evidence require human studies. The evidence must relate to the whole product, a special extract, fraction of same active constituents. The dose form designs and the route of administration, are also important. A claim for a herbal product, requires the herb, the part of the plant, the method of preparation and any processing, the equivalent dry weight and the dose and the active components to be consistent with the evidence used to make the claim.

To many supporters of TCM, the long history of use, tradition, faith, popularity, and anecdotal reports are still the best evidence for the efficacy of TCM interventions. Undoubtedly, convention and popularity provide useful indications of possible efficacy but should not be taken as equivalent to scientific evidence derived from well-designed research. In conventional medicine, the most rigorous method for the evaluation of any medical intervention is the randomized controlled clinical trial (19, 20).

Chapter 4

Difficulties Encountered in Researches on Traditional Chinese Medicine

The distinctiveness of Chinese medicine is manifested in the diversity and the complexity of its components, the instability of its quantity, the fuzziness of its action mechanism, and the uncontrollability of its producing process. The efficacy thus can hardly be guaranteed. For thousands of years, it has been observed by clinical practice that Traditional Chinese Medicine (TCM) has a rich scientific connotation and has developed a unique healthcare system. However, there are problems and challenges that TCM is facing now from the point of view of modern medicine. These challenges include authentication of raw materials, the quality control of herbal products, safety evaluation, and clinical efficacy verification (1-2).

4.1 Uniformity of herbal preparation--standardization of herbs

Traditional Chinese herbal formulae are usually formed by more than one or more plants, animal or mineral items. The composition is extremely complex. The methods of harvesting, drying, storage, transportation, and processing of plant material (for example, mode of extraction and polarity of the extracting solvent, instability of constituents, etc.) influence the efficacy and safety (3). Quality of herbs could directly affect the safety and efficacy of herbal products (4). In order to be accepted by the mainstream medicine, solid scientific evidence is needed to support TCM in quality consistency, and functional claims. However, variable sources of raw materials, unknown active ingredients, difficulties in quality control, lack of safety evaluation,

unclear mechanism of action, etc., all these factors constitute major challenges in modernization of TCM (5).

Quality Control of Herbal Drugs

Compared with synthetic drugs, the criteria and the approach for herbal drugs are much more complex. In general, quality control is requested to answer the following three important questions (6):

1) Identity and authentication: Is the herb the one it should be?
2) Purity: Are there contaminants, e.g., in the form of other herbs which should not be there?
3) Content or assay: Is the content of active constituents or chemical markers within the defined limits?

Well-performed authentication studies could answer the above questions. The authentication of raw materials is critical in research and development of TCM. Plant species must to be identified properly. The strength of pharmacological effects may vary depending on where the plant was grown, when it was harvested, and how long it was stored (7).

Most Chinese herbs are biological products, which include plant and animal elements. The complexity of natural medication is due to its ambiguous ingredients. The differences in quality are influenced by many factors. For example, even the same species their chemical composition may be different, they may be affected by ecological environment, cultivation methods, plant age, soil, climate, sun light, rain and many other factors. In addition, the quality of herbal products is also affected by collecting seasons, preparation methods, and storage conditions. These factors may

make the composition of herbal products vary significantly. The same herb species coming from different sources the quality is inconsistent and results in different effectiveness. In addition, sometimes there are fake herbs that are very difficult to be detected and there are also problems such as mixing with other plants or presence of impurities or contamination of microbe, heavy metals or pesticides. The appropriate control should be given to minimize the bias although some factors that impact the quality of raw herbs are not related to human activities. Therefore the quality control of raw herbs is critical for herbal medicine preparation.

Only when the raw herb itself is standardized, the herbal preparation or herbal products can be standardized. The quality of Traditional Chinese Medicine preparation was rarely concerned in the past. One of the reasons was its historical roots of traditional therapeutic methods and also it was limited by scientific recognition. From the point of view of current recognition, since Chinese medicine is called "medicine", it is necessary to follow the requirements of DRUG standards.

4.2 Verification of herbal formulae

Herbal formula is the fundamental form of Chinese medicine in clinical application. Clinical efficacy and synergistic effects can be achieved by appropriate herbal combination. However, whether the herbal combination is reasonable or not and whether the purposes of reducing toxicity and increasing efficacy are achieved or not, should be scientifically evaluated.

The variety of ancient herbal formulae and the ratio of each herb are the accumulation of experience in the past hundreds of years. Whether herbal formula or the ratio of

each herb is optimal or not, should be proved by using scientific methods. Since ancient times, despite the numerous clinical practices and therapeutic methods that passed through generation by generation, TCM is still lack of convincing evidences in the current view point of evidence-based clinical study. It is not logical to assume that formulations created hundreds of years ago need no change at all.

4.3 Traditional testing of Chinese Medicine

In two century AD in China, a medical Saint *Zhang Zhong-jing* was born, at the same time in the West a medical King *Galen* was also born. However, *Zhang Zhong-jing's* medical system was quite different from *Galen*'s. *Zhang's* theory did not concern the structure and function of human body, and without experimental concept, it only based on syndrome differentiation from the angle of macro observation. In modern medicine, scientific experiments are the basis of truth discovery, and it is also the method of validating the truth. Under regular circumstances, the medical scientific theories should be examined by experiments first, and then validated by clinical researches. Therefore, the experimental study is essential and fundamental. Integration with experimental sciences or not is an important mark of modern science. Apparently, Traditional Chinese medicine does not have such a function. TCM is experience medicine. The authentication of its efficacy and safety came from repeated clinical practices and was then recorded in the books and documents in the form of clinical case reports; it became evidence of efficacy without experimental and clinical validation. Although animal models have been used in TCM researches, it is not easy to create an animal model with "*Zheng*" in Chinese medicine. The study of animal model for traditional Chinese medicine began in 1960 (8). Animal models are usually obtained by repeating the pathogenic causes in the view of TCM. However, from the

perspective of Chinese medicine, the pathogenic factors of Chinese medicine syndromes are complex and non-specific, for example fatigue, disorders of hunger and satiety, emotion irritating caused by common etiologies which including spleen, kidney, heart and liver deficiency. Furthermore, the clinical diagnosis of "*Zheng*" mainly depends on the symptomology and patients' chief complaint reflected in pulse, tongue diagnosis, but animal model cannot repeat the symptoms. Tongue and pulse are also not observed in animal model. Therefore, TCM experimental studies have obstacles; some of them are very difficult to overcome. However, in the process of modernization of Chinese medicine, experimental researches are becoming very important and indispensable; it has played a critical role in the mechanism study of Chinese medicine.

4.4 Concerns of safety and efficacy

Widespread use of TCM brings serious concern about its safety, efficacy, and mode of action (9, 10). In some cases, the use of TCM has been connected to some undesirable side effects (11-12).

Chinese medicine has been used for thousands years and accumulated a wealth of human application experiences. To achieve the desired benefit, adequate amount of dose should be taken. However, there are fewer safety evaluations done with modern scientific methods to study the safety dose. The general opinion believes that traditional Chinese medicine is from nature and has thousands of years' human application experience, so their safety should be guaranteed. The general perception that herbal drugs are very safe and free from side effects is not true. Little do they know in the modern conditions, that the current herbs used for preparation of traditional Chinese medicine have been very different as compared with the ancient

herbs in planting, extracting, producing, storage, application, dose level and the duration of clinical use. The active ingredients of herbal formula are higher after extracting with modern scientific methods; and the toxicity may also be correspondingly higher. The potential side effects after long-term use should not be overlooked. Safety re-assessment is necessary.

For Traditional Chinese Medicine, because of the limitation of the historical conditions, there are no rigorous safety assessments conducted even for many commonly used items. For western medicine rigorous safety assessments are usually performed before marketing. Knowledge of toxicity of TCM is usually from the clinical observation, rather than from well designed experiments. The acute toxicity that occurred within short period is usually casily observed and understood; potential chronic toxicity that leads to organ and tissue damage after long-term use was hard to observe or to be understood in ancient time. As a result, the incorrect concept of "TCM is safe without toxicity" is gradually formed. With the increase of TCM consumption, as well as improper use misled by the concept of TCM toxic free, the clinical toxicity reports presented an increasing trend. For thousands of years, the evidence of TCM efficacy has been accumulated through the case studies. Under the historical conditions, this was an effective way to obtain the effective evidence. With the development of science and the improvement of research methodology, the old methods cannot satisfy the requirements of modern science and technology. In accordance with the principles of randomized double-blind controlled design, TCM medication should be performed for second-evaluation. In addition, the dosage form, dose level, administration route and the duration of treatment may influence the safety and efficacy of Chinese herbal medicine.

Determination of dose, dosage form and duration

Each drug has its proper dose range. Unlike chemical drugs, commonly used traditional Chinese herbal medicines usually have no definitely defined initial dose, maximal dose and toxic dose. Obviously, unduly increasing dose or extending administration duration could induce adverse reactions. Even mild herb such as licorice could induce toxic effects if improperly applied (13).

Chinese Traditional Patent Medicine(中成药) refers to Chinese herbal formula that is prepared according to the principles of TCM theory as a ready-made medicine for easy administration. The dosage form includes capsule, tablet, granular, oral liquid and syrup et al. As the fixed formula and the dosage form, Chinese Traditional Patent Medicine(中成药) can not be as flexible as herbal decoction, which the dose and herbal composition could be adjusted with the disease process.

Dosage forms of TCM are diverse, but no matter how the dosage form changes, two basic characteristics of Chinese Traditional Patent Medicine (中成药) are unchanged: 1) herbal formula and dose are fixed; 2) the fixed formula is targeting a group of population rather than a single individual.

What kind of dosage form and how high the dose is safe and effective? This is an important issue for TCM research. Traditional Chinese Medicine is lack of dose concept such as the three bowls of water decocting into a bowl of decoction and also without the duration of drug administration. Dose typically includes two implications: the amount of a single dose and the duration of the same dose to be taken. For general TCM medications, these two points are blurring. In TCM dose-finding researches, it is hard to find out a clear dose-response relationship between dose level and therapeutic effectiveness. This is perhaps due to the variety and complexity of TCM ingredients that make the linear relationship between the dose and effects not so clear. In order to ensure the effectiveness of the TCM drug, it is necessary to find out an optimally effective dose for clinical application.

Herb- drug interactions

Mixing TCM with Western medicine may produce more toxic effects or synergistic effects (14). TCM medications could also affect the metabolic rate of Western medicine (15).

Combined use of herb with drug may increase or reduce the effects of either component (16-17). Synergistic or additive therapeutic effects may lead to unfavorable toxicities and complicate the dosage regimen of long-term medications, while antagonistic interactions could result in decreased efficacy and therapeutic failure. The potential interaction of herbal medicines with drugs is a major safety concern.

The potential of the integration of Chinese and modern medicine in future medical practice must not be underestimated with the use of herbal medicines widespread and increasing. Hence, the unavoidable interaction between Chinese and modern medicine needs to be understood. Will such combination therapies enhance or worsen the treatment outcome, or even be harmful? This is the question waiting to be answered.

It has been found that many Chinese medicine or modern drug therapies are only beneficial to a patient if they are given at certain dosages. When they are used at other dosages instead, they turn out to be noxious.

The famous Chinese doctor *Zhang Zhi He* (张子和) of the *Yuan Dynasty* (about 1300 AD) had once wrote in Enlightening Case Reports (*Ru Men Shi Qing* 儒门事亲) (18) "Toxicity is a property of all drugs — not just that poisons are toxic. One must not forget herbs such as *Liquorice* (*Radix Glycyrrhizae*) and *Radix Sophorae Flavescentis* which can be toxic too. Undesirable outcomes follow any type of over dosages." Both toxic and curative potentials are properties of all drugs. The key to successful

treatment is to select the right drug, the right dosage and the right administration route. For every drug including TCM, there is a direct relationship between its dosage and the severity of its toxicity or side effects. The interaction of Chinese and modern medicine may be synergistic; it may also be antagonistic, or even be noxious. Simultaneous practice of Chinese and modern medicine may complicate the control of dosages of any patient's long-term medications. It has already been reported that the traditional Chinese medicine for diabetic control, if used with insulin injection or oral anti-diabetics, may cause hypoglycemia (19).

The interactions between Chinese herbs and modern drugs are a common issue, yet reports on them are very rare. According to research on 1000 elderly patients admitted through the accident and emergency department, 538 of them have used over 1087 types of drugs, and 30 of them have been affected by the side-effects of these drugs (20).

Some drug interactions have been investigated by *in vitro* and *in vivo* experiments, but results obtained have been inconsistent. *St. John's wort*, an herb commonly used in Western societies, was shown to suppress monoamine oxides *in vitro*, but such observations were absent *in vivo* studies.

Appropriate integration of Chinese and modern medicine may have synergistic effects such that treatment outcomes are enhanced and side-effects are suppressed. For example, in the case of tonsillitis, co-administration of *Radix Isatidis* (*Banlangen*) with Trimethoprin (TMP) significantly enhances the immune system; the outcome may be much better than administration of either drug alone.

Conversely, disastrous outcomes may follow inappropriate usage of Chinese and modern medicine: antagonistic interactions resulting in reduced or loss of curative potential, enhanced side-effects, or even death. For example, co-administration of digoxin and *Liu Shen Pill* (*Liushenwan* 六神丸) led to repeated ventricular

extrasystoles; mixing *Cinnabaris* (*Zhusha*) containing Chinese Medicine with halogen compounds can be extremely toxic (21).

Some herbal medicine, even when used alone or combined, should not be overdosed. Children and those with ill health, if overdosed on *Aloe vera*, present with hypersensitive reaction — erythema, psoriasis, and even nausea and diarrhorrea-like symptoms. Pregnant women and those menstruating should not use Aloe vera since it may increase the vascularity of the female organs, stimulate uterine mobilities, and cause epigastric pain and severe bleeding. Patients suffering from haemorrhoids and epistaxis could suffer from enhanced symptoms by taking *Aloe vera* (22).

Determination of the duration of therapy

How long the Chinese medicine should be taken to achieve the expected results without toxic effects has been the problem in front of Chinese medicine research. As the efficacy of Chinese medicine comes out slowly, if the duration of administration is too short, the therapeutic effect might not be reached. Conversely, if the duration of administration is too long, the toxic effects may occur.

An excessively higher dose or longer duration of treatment than that used historically and traditionally may cause safety concerns because the usefulness and relevancy of previous human experience with the herbal to the proposed human protocol were diminished and resulted in requests for modification. It is very critical to determine the optimal dose and duration that could balance the safety and effectiveness.

Long-term safety issue

During the past one hundred years, there were many major global drug safety incidents in the world (23). These incidents bring people to the awareness of the

importance of the safety issue. For example, the incident of "sulfa-anhydride agent" happened in the United States in 1930s made 107 people died due to no animal safety tests were done before clinical trials. During the same period in the United States and Brazil, many people used diet pill dinitrophenol to control weight. As there were no long-term and comprehensive safety evaluations, patients suffering from cataract greatly increased after 20 years later of taking the drug. There was a well-known event which occurred in 1960's of the last century, i.e. "thalidomide" event. Thalidomide was a medication used to treat gynecological pregnancy reaction. Due to no teratogenicity tests performed, 8000-10000 infants developed congenital malformations. These events caused by chemical substances has led researchers and relevant authorities to pay considerable attention to the safety and evaluation of chemical drugs, however, little attention was paid to the safety of Chinese herbal medicine. Although the human application of traditional Chinese medicine has been 5,000 years and it was generally believed safer than Western medicine. In fact the safety data of TCM evaluated by using modern scientific methods is very limited. We do not even know the basic information of some commonly used TCM such as the safety dose, therapeutic duration and maximum tolerant dose.

The logic of skipping toxicity tests in TCM research is probably based on an assumption that Chinese herbal preparations have been used safely for centuries, therefore a special toxic screening is not necessary. We have strong reservations on this attitude and would recommend that toxicity clearance should remain the first phase of clinical trial (24).

For example in Allergic Rhinitis (AR) clinical trial, patients with allergic rhinitis were treated by AR capsule.

AR capsule is a modified ancient classic herbal formula, which contains Cangerzi (苍

耳子). For safety reasons, the long-term toxicity should be evaluated before conducting a clinical trial.

The objective of long-term toxicity study is to assess the chronic and systemic toxicity of study TCM in the rat when administered for a period of 30 days, 60 days or 90 days according to the duration of clinical application.

The rat is used as the test model because of its proven suitability in toxicology studies. Oral treatment is chosen in order to comply with the intended clinical route of most TCM preparations. The dose levels are usually selected based on the recommended clinical daily dose. Some details of the necessary steps are given below to serve as an example.

Forty five male and 45 female rats were used, 30 animals per gender for the control and each dose group.

At the start of the acclimatisation period, the rats were 5 to 6 weeks old and in the weight range 150-160 g. Throughout the study, rats were kept in transparent polycarbonate cages with twenty to thirty animals in each cage, males and females separated. The study took place in the room at a temperature of $25^0C \pm 2^0C$, relative humidity of 60% ± 15%. Rodent diet and filtered sterile drinking water were available *ad libitum* during study period.

All rats were examined daily for mortality and clinical signs. Body weights were recorded on arrival, on the first day of treatment (day 1) and weekly thereafter.

At the end of 30-day, 60-day and 90-day study period, hematology and clinical chemistry were performed on all surviving animals. Parameters evaluated were the following:

Hematology blood cell morphology, erythrocyte indices, hematocrit, hemoglobin, mean platelet volume, platelets, red and white blood cells and reticulocytes counts

Clinical chemistry albumin/globulin ratio, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, blood urea nitrogen, cholesterol, creatinine, electrolytes, globulin, glucose, total bilirubin and protein

This long-term toxicity test could tell us the safe dosage range and safe administration duration, and adverse reactions if any. Based on the animal toxicity study results we can estimate suitable human dose for further clinical trial.

4.5 Randomized Controlled Clinical Trial

Evidence-based medicine emphasizes that all medical practice must be based on the best available evidence. Randomized controlled trial (RCT) is the generally accepted scientific research method in the efficacy evaluation of an intervention, and the results of which could provide the basic evidence for efficacy evaluation.

The use of Traditional Chinese Medicine in China has experienced several thousand years. In spite of established systematic theories and unique treatment methods, it is still lacking proper clinical efficacy evaluation. Controlled clinical trials are scarce. Evidence was simply based on the patient's subjective control of symptoms to judge whether a disease is severe or improved. Accumulated clinical experiences were recorded in ancient medical documents in the form of medical case reports, which emphasized on the improvement or disappear of the symptoms and used them as a standard to determine the clinical efficacy. Restricted by the historical conditions and recognition at that time, the clinical practitioners then could only be able to do so was understandable, but the reliability and scientific implication needs to be improved. With the current standards of evidence-based medicine, the efficacy re-evaluation on Chinese herbal medicine is imperative. Evidence-based medicine approach is a

double-edged sword. It can verify the effectiveness of traditional Chinese medicine and its scientific nature, but if improper methods employed it may also fundamentally reject Traditional Chinese Medicine.

Traditionally, the clinical research of Chinese medicine primarily depended on the individual physician. Individual mode of production, scattered distribution in urban and rural areas made the clinical conclusions having more intense individual experiences. These results with the universal, from a methodological point of view, were lack of the basis of identity and recognition, and it may be one of the reasons why the different Schools of Thought of TCM are so different. In such a repeated process that theories guide the clinical practice, vice versa, clinical practices validate the theories, and many valuable experiences were concluded. Overall, these experiences have their own positive sense, but for a single individual, it is hard to distinguish their true value. Some experiences may have a pattern, but some experiences may only have individual significance without a common pattern, and some may be wrong. How to determine the value of these experiences, identify its usefulness, and there is only one answer: performing well designed clinical trials in line with the recognized guidelines and obtaining validated evidences to support the claimed efficacy, only in this way, the experiences can be upgraded to the regulation level and accepted by mainstream medicine.

Proprietary Chinese medicine made from a herbal formula is targeting to a group of patients, its efficacy does not only meet the requirements of a small number of patients, but it is also effective to a group of patients. Therefore, research methodologies used to evaluate the clinical efficacy of herbal formula in large population must be adopted. Randomized, blind and controlled clinical trial as golden

standard for efficacy evaluation has been widely recognized, it is also equally applicable for TCM study.

Blinding can be a real problem in "double-blind" trials of TCM. Due to taste, odour or appearance, herbal medicines may be distinguishable from their respective placebos (25). Un-blinding can therefore be a problem and exert an undue influence on the clinical results. For instance, there are numerous "placebo-controlled, double-blind" trials of DBT preparations for postmenopausal symptoms. But anyone who has ever been involved in such a study knows that, due to the body odour caused by *Danggui*, blinding is not a realistic option. For clinical studies of acupuncture, blinding is, of course, a significant problem. (26).

In general, many factors may influence the results and efficacy consistency of clinical trials:

- Lack of standardization and quality control of the herbal drugs used in clinical trials
- Use of different dosages of herbal medicines.
- Inadequate randomization in most study and patients not properly selected
- Numbers of patients in most trials are insufficient for the attainment of statistical significance.
- Difficulties in establishing appropriate placebo because of the tastes and aromas.
- Wide variation in the duration of treatments using herbal medicines.

Due to such difficulties, few herbal formulae have been studied comprehensively and adequately and well-controlled double-blind clinical trials for proving their safety, efficacy have been lacking.

4.6 The problems of dose determination

Clinical trials of traditional Chinese medicine faced by the challenges of how to determine a reasonable initial dose, how to determine the administration interval. Because classical traditional Chinese herbal formulae have been supported by TCM theories and clinical experience, basic understanding of their tolerance and clinical experience were usually obtained. Pre-clinical studies such as tolerance and pharmacokinetics were no need to be performed. If there were no data of "Phase I clinical trial," and relevant reference, and without previous persuasive clinical data, how to recommend effective clinical dose? If the dose-effective relationship is not clear, how to determine the drug dose and administration interval? Therefore, considering the unique characteristic of traditional Chinese medicine the exploration of clinical dose is an important issue that is worthwhile to study.

4.7 Recruitment and Compliance

Failure to recruit sufficient numbers of participants is a major barrier to the completion of randomized controlled trials in traditional Chinese medicine (TCM) clinical trials. Efficient and effective recruitment of patients and retention of participants are essential steps in the clinical trials. The difficulty of recruitment depends on the exclusion/inclusion criteria, age, study medication, disease risk factor, biomarkers tested, stage of disease, etc. The barriers to recruitment also came from fear, distrust, or misunderstanding of the clinical trial process, unwilling to take herbal medication, unacceptable to some ingredient(s) of study TCM formula or the fear of being treated like a "guinea pig".

For some patients, the barriers would come from time constraints, travel problems,

worry about overdoing the blood tests, too many visits scheduled, instead of an active medicine. Insufficient recruitment is also due to Western doctors' unsatisfactory attitude to TCM study.

Another important issue in clinical trial is compliance. Compliance in clinical trials includes two meanings: 1) the deviation from the original design scheme; 2) the integrity of the treatment and follow-up visits. Compliance is one of the important indicators in evaluation of the quality of a randomized controlled clinical trial. Compliance includes the integrity of a recruited patient in receiving the therapies, drop out or lost follow-up cases and the reasons.

The compliance of TCM clinical trials has their special difficulties. Subjects may take other kind of TCM or functional food that was similar to the study medication. As many TCM medications or functional food are easily available in the market, many subjects taking part in the clinical trial may take them on their own. In particular, in randomized double-blinded placebo-controlled clinical trial, subjects worried about to be assigned to placebo group usually purchase the similar product from the market. We have conducted a randomized double-blind placebo-control clinical trial on patients with breast cancer, more than 95% of the subjects took the herbal drug similar to study medication. There is no doubt this would lead to the deviation of results (27).

4.8 Statistical analysis

Appropriate statistical methods for analyzing clinical trial data are critical for the correct interpretation of the results. Statistical considerations, appropriate to the design of the trial, including sample-size calculations, timelines for any interim analyses and a sketch of a proposed statistical plan for analyzing these endpoints,

should be detailed in the statistical section of the protocol and reported in subsequent publications (28). The analysis principle for the primary outcome should be that of intention-to-treat, where the data are analyzed according to the treatment group to which they were randomized (29).

Data analysis is critical in TCM clinical trials. If the valuable clinical trial data were improperly analyzed, not only we might get incorrect conclusions, but also mislead the researchers and make actually effective TCM drug become ineffective or actually ineffective TCM drug to effective. The former results will waste research resources, while the latter may delay the treatment time, and result in serious consequences. Therefore, the correct statistical methods for data analysis are very important. In the course of TCM study, clinical trial conducted according to the scientific principles is the only reliable foundation in evaluation of the efficacy and safety of TCM. For a study medicine, if clinical trial cannot properly evaluate its safety and efficacy, the clinical trial might be a failure. Many factors can lead to clinical trial failure. Some of the factors are controllable, while others may be difficult to control. Clinical trial design plays a vital role for the success of clinical trials. Improper hypothesis and randomization and blinding methods, inclusion/exclusion criteria too strict or too loose, the baseline of subjects too variable, improper treatment dosage and endpoint selection, the sample size too small and employment of inappropriate statistical analysis method, all of these factors may lead to a clinical trial failure.

Importance of sample size estimation

In theory, to validate a difference of an intervention between control and study groups, the larger sample size, the results more close to the true value, i.e., the more reliable the results. However, due to the resource and ethical constraints, the number of subjects in a clinical trial can not be infinite, it is necessary to determine the optimum

sample size according to the requirements of statistically significant test.

The size of the expected effect of the intervention is the main determinant of the sample size necessary to conduct a successful randomized controlled trial. Obtaining statistically significant differences between two samples is easy if large differences are shown. However, the smaller the expected effect of the intervention, the larger the sample size needed to be able to conclude, with enough power, that the differences are unlikely to be due to chance (30).

For example in our previously completed osteoporosis clinical trial, we wished to study two groups of patients who would undergo different interventions, one of which was an herbal formula and another was placebo. We expected a mean annual decrease in spinal BMD of approximately 1.9% in untreated women. The actual SD in our previous study was 3.6%. To detect a 1% difference between the two treatments in spinal BMD using a two-sided 0.05 α-level test with 90% power required 136 subjects. If the expected difference in effect between the two groups increases to 2%, the number of patient required decreased to 34. Conversely, if the difference between the groups was expected to be only 0.5%, the study population must increase to 544.

4.9 Outcome measurement in TCM clinical study

The gold standard for measurement of the efficacy of single drugs may not be the best research tool to measure the efficacy of TCM where many herbs may be used in herbal prescription and each disease episode and every patient is treated as being different from any other. A change in perspectives is required which facilitates research methodologies that understands and respects the integrative and holistic nature of TCM. Treatment of TCM is aimed at restoring the balance or harmony of

the whole person –psychological, social, physical and spiritual.

The purpose of clinical trial is to verify the efficacy and safety of an intervention. Selecting what kind of efficacy parameters (or endpoints) is an important issue in clinical trial design (31). The choice of outcome parameters is critical for the efficacy evaluation of an intervention. It is directly related to the success or failure and reliability of a clinical research. Efficacy parameters should be sensitive, specific and economically feasible. Evidence-based medicine specially emphasize on endpoint or outcome parameters)。

Currently, the problems of TCM clinical trial in the issue of efficacy evaluation include no unified assessment standards, not aligning with international standards, improper choice of endpoints, and inappropriate statistical methods. For example, the treatment of chronic viral hepatitis with Chinese herbal medicine, the efficacy parameters under the "*Efficacy evaluation of viral hetatitis treated with Chinese medicine*" (China Association of Traditional Chinese Medicine Internal Medicine Professional Committee of liver disease 1991), is divided into basically clinical cure, effective 、 improved and failure. These standards can not be in line with international standards because of its poor accuracy and objectivity, the investigators have their own subjective with larger variability.

Outcome measures used in clinical trials should be validated. In trials of TCM, soft and non-validated outcome measures are often employed, e.g., percentage of patients perceiving benefit or patients' preference. Similarly, multiple outcomes are frequently used without adequately accounting for multiple statistical tests. Finally, surrogate endpoints are frequent and researchers often seem to measure what is measurable rather than what is relevant.

In the outcome or endpoint of TCM clinical trial, the issue of "*Zheng*" is more prominent, mainly reflected in no strict diagnostic criteria, it is too simple and too random in quantitative of *Zheng*. The evaluation of efficacy based on *Zheng* is too subjective.

Biomarkers and Surrogate Endpoints

Over the last 50 years, biostatistics has provided a framework for designing and analyzing clinical investigations to determine the clinical benefits of a treatment as well as to determine its effects on biomarkers of health or disease status.

There has not been a consistent use of terminology in the scientific a ndmedical literature describing the substitution of biological parameters for clinical endpoints. Recently, a National Institutes of Health (NIH) working group recommended preferred terms and definitions that have broad applications (32):

Biological Marker (Biomarker): A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

Clinical Endpoint: A characteristic or variable that reflects how a patient feels or functions, or how long a patient survives.

Surrogate Endpoint: A biomarker intended to substitute for a clinical endpoint. A clinical investigator uses epidemiologic, therapeutic, pathophysiologic, or other scientific evidence to select a surrogate endpoint that is expected to predict clinical benefit, harm, or lack of benefit or harm.

Surrogate parameters for clinical trial

Outcome assessment is currently a hot research field as it is increasingly recognized

that adopting appropriate parameters to evaluate efficacy is an important part in clinical trials. Using different parameters for efficacy assessment could draw diametrically opposite conclusions (33).

A surrogate outcome in the context of clinical trials has been defined as 'a laboratory measurement or a physical sign used as a substitute for a clinically meaningful end-point that measures directly how the patient feels, functions or survives. Changes induced by a therapy on a surrogate end-point are expected to reflect changes in a clinically meaningful end-point.' (34). The benefits of choosing to study surrogate variables instead of primary outcomes in clinical trials are that they may shorten the period of study, lower the sample size required and lower the costs of the study. Ideally, if there is a strong correlation between change in the surrogate variable and the primary clinical end-point (i.e. cardiovascular event rates, cancer survival rates, pregnancy rates), the effectiveness of therapy on the primary end-point can be quantified by estimating the proportion of the treatment effect explained along with its standard error (35-36).

Nowadays, there are problems in efficacy evaluation of TCM clinical trials, which include un-unified assessment standard, not in line with the international standards, improper parameters and inappropriate statistical methods etc.

4.10 The contradiction between TCM and Modern medicine

In Clinical trial design, it is critical to take into account the feasibility of implementation. Otherwise divorcing the plan from reality and acting blindly may result, finally the design has to be modified, or the clinical trial may be withdrawn.

One of the biggest differences between TCM and western medicine is the non-specific nature of TCM treatment. The mode of treatment modalities of TCM is a holistic

approach. In design of a TCM clinical trial, it is difficult to use a single endpoint to assess its effectiveness, but using multiple endpoints may increase the difficulty and reduce the feasibility of implementation. In the clinical study of western medicine, the conventional approach is a clinical study aiming at only one target. The design is straightforward and the method is directed towards one outcome. In Chinese medicine, the study is much more complex. In order to satisfy the holistic approach and fully evaluate the efficacy of traditional Chinese medicine which has many efficacy claims, single endpoint cannot meet the multiple-claims. In the patient recruitment, in addition to a perfect adopting of the inclusion/exclusion criteria, it is also necessary to remove other influencing factors like whether the subjects were taking other related Chinese herbal medicine. Outcome offer require an assessment of the quality of life. Design of TCM clinical trial is therefore more complex, which affects its implementation and operability.

4.11 Chemical reference substances and standard materials

Chemical marker of Chinese herbal medicine (CHM) is derived from the concept of chemical reference material. Due to the fact that Chinese medicine is not yet generally accepted as a pharmaceutical medication, international chemical marker only contains plant chemical references. China is the first country to establish a collection of state-level CM chemical standard markers.

It is a main challenge to establish a quality control system of Chinese herbal medicine for the clinical effect and safety, which would be accepted by the international pharmaceutical industry. In respect to the ingredient assay, always only 1 or 2 marker ingredients are determined, which is not in accordance with the complex properties of Chinese herbal medicine. It becomes difficult to evaluate in whole the qualities of

Chinese herbal medicine and their products. The fingerprinting of Chinese herbal medicine is a quality control method that is integrative and quantified.

The complex properties of Chinese herbal medicine always include multiple chemical ingredients in Chinese herbal medicine, which can collaborate and aim to different targets when they work. Moreover, only by considering the items such as the description, physicochemical identifications, and the assay, the specificity of each Chinese herbal medicine is poor.

Chemical markers not equal to Biological activity

Chinese medicine is usually monitored by the chemical marker approach using a few characteristics of chemical components. The markers may or may not be the active ingredients. These chemical markers are usually taken as reference standards in chromatographic analysis via HPLC and GC-MS, which are widely used for Chinese medicine quality control.

Chemical markers of Traditional Chinese medicine cannot fully control the quality of traditional Chinese medicine. In the past, a lot of studies have been conducted in quality control from the angle of physical and chemical properties by using chemical markers as standards, especially from the angle of chemicals rather than the angle of safety and efficacy. Therefore, the quality standards of most of traditional Chinese medicine can only control the physical and chemical properties and part of the biological activity (chemical composition), but cannot completely control the safety and efficacy of Traditional Chinese Medicine because these chemical markers may not contain active ingredients.

China government has appointed the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, 中国药品生物制品检定所) to

demarcate and provide CM chemical markers. However, there are only less than 400 kinds of CM chemical markers available, and the quality and marketization are still flawed, this make the development of the quality control of Chinese medicine to be confined.

According to partial researches, apart from NICPBP all listed types provided by other 54 enterprises are limited and their quality is inconsistent. Since they are non-authorized suppliers, their CM chemical standard references are limited in scope of usage (37). The types of CM chemical markers announced by the state exceed 900, but available types are merely around 400. Under this circumstance, a large number of TCM pharmaceutical products requiring the testing of CM chemical markers actually cannot be analyzed.

Chapter 5

Overcoming the Difficulties---Quality control

In the past 60 years, science and technology have made rapid development, which greatly enriched and improved the methodology of TCM research. TCM is originated from clinical practice and has experienced several thousands years. During the long course, experiences and recognition of TCM were accumulated. The theoretical foundation of TCM in healthcare, prevention and therapies were gradually formed. Traditional efficacy evaluation mainly came from the practitioners' experience and patients' subjective feeling. In addition to subjective factors of physician and patient, the stability and consistency affect the efficacy of herbal medicine. Therefore, the research of TCM should start from the quality control of starting raw herbs.

5.1 Quality control of Chinese herbal medicine

The primary objective of randomized controlled trials (RCTs) is to investigate the efficacy and safety of specific interventions in health care improvement. To achieve the purpose, one of the most important preconditions is the quality of study herbal medicine that should be having batch-to-batch consistency. Quality control for Chinese herbal medicine includes species identification, location of production, collection, processing methods, and screening for contamination.

The clinical effects of herbal medicine come from the contained chemical components. A medicinal herb with different names or different origins and its homologous

products may have different chemical components that would lead to distinct pharmacological and clinical effects. More seriously, some of them are invalid or toxic. Therefore, the authentication of medicinal herbs is directly related to their clinical effect and safety. We take the authentication of *Hedyotis diffusa.* (HD 白花蛇舌草) and *Hedyotis corymbosa (L.)* (HC 水線草) as example to elucidate the importance of authentication.

Hedyotis diffusa. (白花蛇舌草) is widely distributed in subtropical areas. The herb is considered to be latent-heat-clearing, antipyretic, anti-inflammatory, detoxicant, blood-stimulant, and anti-carbuncular. It is mainly recommended in carbuncles, sores and chronic ulcers, pharyngolaryngitis, pathogenic "damp-heat" in the pelvic region, and externally in the treatment of injuries due to impact, fractures, contusions and strains, and in poisonous snake bites. A related species, *H. corymbosa (L.) Lam.* (HC 水線草) morphologically mimics HD, is a folk medicine. HC is often used as HD in folk medicine. HC has been reported as the major plant that is confused with HD, and such confusion seriously compromises the consistency of the quality and the therapeutics of HD. Our studies have demonstrated significant chemical differences between HD and HC, and intermixing has the significant potential of compromising the therapeutic benefit and the research of HD (1). These authentication procedures make sure that the herbs we used in our study medication are what we want.

The quality control of Chinese herbal medicine is a systematic procedure. The initial and critical step is to standardize starting raw herbs, for example the exact species and subspecies, ideal growing location, environmental conditions, harvesting methods, and storage conditions, to ensure the quality of the raw materials. The second step is to standardize the processing methods of the raw materials produced from herbs according to Good Agriculture Practice (GAP) guidelines. The third step is to

standardize the preparation procedure of the final products according to the requirements of Good Manufacture Practice (GMP) guideline. The fourth step is to qualitatively and/or quantitatively evaluate the quality of TCM medication based on one or more selected chemical markers.

Theoretically, the purpose of standardization is to ensure that each dosage unit of herbal drug product delivers the same amount of active ingredients. As herbal medicinal products are complex mixtures that originate from biological sources, great efforts are necessary to guarantee consistency in quality.

Standardization as the basis of modernization and internationalization of TCM is the key issue to ensure the safety and efficacy of TCM products. Lack of standardization in TCM products impedes the development of TCM. Some herbs cultivated in different regions or harvested in different seasons may vary considerably in their chemical and biological properties. Most of the TCM products do not have specific biomarkers. Only about 500 Chinese herbs have their standard chemical references identified (2).

Table 5-1 Medicinal materials recorded in the Chinese Materia Medica

Resource	Family	Genus	Species	Percentage (%)
Medicinal plants	383	2,309	11,146	87.0
Medicinal animals	395	862	1,581	12.3
Medicinal mines	--	--	80	0.63
Total			12,807	

More than 11% of the world plant species are found in China, including 240 rare genera. A national survey indicated that China had 12,807 species of medicinal materials (**Table 5-1**) (3), in which 11,146 species (9933 taxonomic species and 1213

taxonomic units under species) are medicinal plants, including 10,687 species of seed plants, bryophytes, or pteridophytes and 459 species of algae, bacteria, fungi, or lichens (**Table 5-2**).

Table 5-2 Medicinal Plants Resources in China

Resource	Families	Genera	Species
Algae	42	56	115
Bacteria	40	117	292
Fungi	9	15	52
Lichens	21	33	43
Bryophytes	49	116	456
Pteridophytes	222	1,972	10,188
Seed plants	383	2,309	11,146

Authentication and quality control of Chinese medicine preparations and Chinese medicinal herbs has been a challenge to scientists in medical and industrial fields. A major problem is that the plants are very complicated: they contain multiple chemicals with variable biological effects. This multi-component system makes the identification of chemical markers and active ingredients of the Chinese medicine very complicated and difficult.

Danggui Buxue Tang (DBT) will be used as example to elucidate the standardization of herbal formula composition. Among thousands of TCM formulae, *Danggui Buxue Tang* (DBT) is one of the simplest. The formula consists of only two herbs: *Radix Astragali* (RA, Huangqi 黃芪) and *Radix Angelicae Sinensis* (RAS, Danggui 當歸) in a weight ratio of 5:1. According to a traditional method, the herbs are boiled together in two bowls of water at moderate heat until the final volume has been reduced to one bowl [4]. In a ancient book entitled *Neiwaishang Bianhuo Lun* (內外傷辯惑論) in 1247

AD, DBT was first described by *Li Dongyuan* (李東垣), who was one of the four well known TCM physicians during the *Jin and Yuan Dynasties* in China. Since then DBT has been used for around 800 years.

A reliable and reproducible chemical composition is a prerequisite in the maintenance of batch-to-batch consistency of Chinese medicine preparation. The quality of *Radix Astragali* (RA) (*Huangqi*) and *Radix Angelicae Sinensis* (RAS) (*Danggui*) may be considerably influenced by weather, geographic location, soil conditions, and the methods of cultivation and processing. Some Chinese medicinal materials with excellent quality are only produced in certain regions of China which are often referred to as '*the best growth region*' or '*Daodi*' (道地). Therefore, how to authenticate and choose the best *Radix Astragali* (RA) and *Radix Angelicae Sinensis* (RAS) becomes a critical element in ensuring the quality of DBT.

Astragalus L. (Leguminosae) is a large genus which has over 2,000 species worldwide and more than 250 sections in angiosperm family Fabaceae (subfamily Papilionoideae). Both listed as the botanical sources of RA in Chinese Pharmacopoeia (2005) (5). *Astragalus* membranaceus (Fisch.) Bunge and *Astragalus* membranaceus (Fisch.) Bunge var. mongholicus (Bunge) P.K. Hsiao are the most commonly used RA (6, 7).

Isoflavonoids, astragalosides, polysaccharides, amino acids and trace elements are contained in *Danggui* and *Huangqi*, which serve as chemical markers. HPLC and spectrophotometry are used to determine the levels of them in different seasons and various ages. The results indicated that RA of three years of age from *Shanxi*, China contained the highest amounts of isoflavonoids, saponins and polysaccharides (8, 9).

According to the Chinese Pharmacopoeia (2005) (5), RAS is the root of *Angelica sinensis* (Oliv.) Diels (family Umbellaceae); however, *Angelica acutiloba* (Sieb. et Zucc.) Kitag. and Angelica gigas Nakai, mainly found in Japan and Korea respectively,

are also sold as RAS in the markets of South East Asia (10, 11 ,12,13). Studies have shown that the three commonly used Angelica roots vary in their chemical composition, pharmacological properties and efficacy (14 ,15).

The main chemical constituents of Angelica roots are ferulic acid, Z-ligustilide, angelicide, brefeldin A, butylidenephthalide, butyphthalide, succinic acid, nicotinic acid, uracil and adenine (14 , 16 , 17 , 18). The levels of ferulic acid and Z-ligustilide are often used as chemical markers for the quality control of Angelica roots (17). In A. sinensis roots from *Gansu*, China, the levels of ferulic acid and Zligustilide are about ten-fold higher than those of the roots of A. acutiloba (from Japan) and A. gigas (from Korea) (14, 18). *Su Jing* (659 AD) in *Tang Bencao* (苏敬 唐本草) and *Li Shizhen* (1596 AD) in *Bencao Gangmu* （李时珍 本草纲目） recorded that *Angelica* roots of two years of age produced in *Gansu* were the authentic source. RAS from *Gansu* contains about two-fold higher amounts of Z-ligustilide and ferulic acid than those RAS from *Yunnan*, *Shanxi* or *Sichuan*, China.

To ensure the best quality of DBT decoction, the standardized RA from *Shanxi* and standardized RAS from *Gansu* were used in our DBT preparations (19).

The demand for high quality, safe, effective, and uncontaminated herbal products have been growing significantly. In the past, medicinal herbs were largely harvested from the wild and brought to the market without any question asked about their origin, methods of cultivation, botanical identity, purity, safety, and efficacy. The quality of medicinal herbs has traditionally been based on appearance and experiences. An important early visual quality evaluation was to ensure that the herb was of the required species. The medicinal efficacy of many herbs varies greatly between herbs of different species of same genus.

Chemistry-manufacturing-control (CMC) considerations for herbal products

Unlike chemically-defined drugs, herbal products have had substantial human use prior to clinical trial evaluation. Evaluation of herbal products does not require attempts to purify the medicines down to known or otherwise single chemical constituents. For herbal products, "analysis of the active pharmaceutical ingredient(s)"may be best approached by analysis of one or more hypothesized active ingredient(s), analysis of a chemical constituent that constituents a sizable percentage of the total ingredients, and a chemical fingerprint of the total ingredients. Variation of content from batch to batch is an important issue for herbal products. Several analytical procedures are needed to adequately quantify their constituents (20).

Recently, the problems concerning the quality control of herbal products have gained public attention. Due to the complex chemistry and the great variation of the medicinal herbs, it is a big challenge to control the quality of each herbal material on the market with the current analytical technologies. The ultimate requirements of drugs should be efficacy and safety, whereas the elucidation of ingredients and mechanisms, though not a must, would be helpful. Consistency of quality among different batches of herbal drugs is a prerequisite for the use of herbal medicines (21).

Recent approaches in herbal medicinal standardization

The quality control standards of various medicinal herbs are becoming more relevant today in view of industrialization. For standardization and quality assurance purposes, three attributes are desirable:

1) Authenticity,

2) Purity and

3) Assay.

Authenticity relates to proving that the material is true. Authentication in itself involves many parameters including gross morphology, microscopy, chemical analysis and DNA fingerprinting. Purity pertains to evaluating that there are no adulterants present in the herbal material. Assay part of standardization is chemical and biological profiling which could assess the chemical effects and curative values established (22).

Chromatographic fingerprinting

In recent quality control systems of Chinese herbal medicine, chemical fingerprinting is a powerful technology in authentication of Chinese herbal medicine.

In terms of methodology, the technology of fingerprinting of herbal medicine mainly includes: 1) Chromatography (e.g. thin-layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC), and supercritical fluid chromatography (SFC)). 2) Spectroscopy (e.g. ultraviolet spectroscopy (UV), infrared spectroscopy (IR), and nuclear magnetic resonance spectroscopy (NMR)). 3) Other hyphenated technologies (GC-mass spectrometry (MS), LC-MS, LC-NMR, and LC-diode array detection (DAD)-MSn). Among the above, chromatographic fingerprinting is the mainstream method.

Chromatographic fingerprint analysis is used for determining the identity, stability, and consistency of Traditional Chinese Herbal Medicine (TCHM) as well as the identification of adulterants.

In the chemical analyses of *Radix Angelica* (*Danggui*; 當歸) in DBT, the main constituents of *Angelica* roots (*ferulic acid* and *Z-ligustilide)* were served as chemical markers for the quality control. HPLC and spectrophotometry were used to determine the levels of *ferulic acid* and *Z-ligustilide* (17).

The roots of *A. sinensis* showed a clear distinction that contained ~10 folds higher of

ferulic acid and *Z-ligustilide* as compared to roots of *A. acutiloba* (Korea Danggui) and *A. gigas* (Japan Danggui). The amounts of main constituents in roots of *A. sinensis* varied according to different regions of cultivation and different methods of preservation. The best source of *Danggui* is from Gansu of China. **[Figure 5-1]** (23)

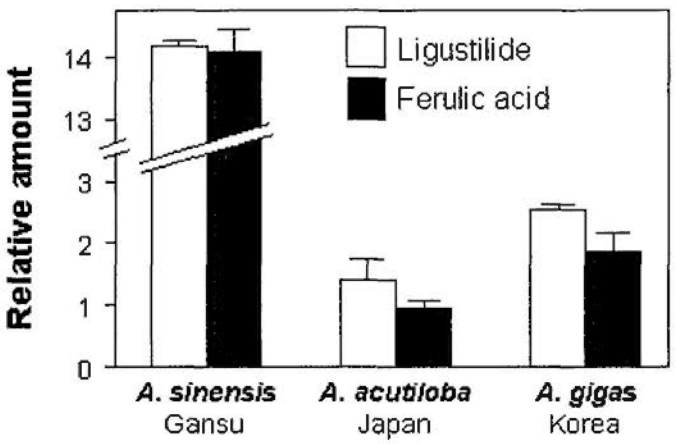


Figure 5-1. Contents of *ferulic acid* and *Z-ligustilide* in different species of Angelica roots

DNA fingerprinting

Genetic makeup inspections provide a definite answer to the botanical identity of the TCM, as the genetic makeup of an herbal species does not vary with their physical form, physiological and external conditions.

Astragalus L. (Leguminosae) (*Huangqi*; 黃芪) is a large genus with over 2,000 species worldwide. The botanical sources of *Huangqi* in Chinese Pharmacopoeia (2005), which include *Astragalus membranaceus* (Fisch.) Bunge and *Astragalus membranaceus* (Fisch.) Bunge var. *mongholicus* (Bunge) are the most commonly used species. The morphological appearances and chemical properties of *Huangqi* and its adulterants show a remarkable resemblance [8]. The DNA sequences of 5S rRNA spacer, ITS and 18S rRNA coding region were determined and compared among ten

Astragalus taxa [22]. The common substitute of RA in some parts of China is the roots of *Hedysarum polybotrys* which has very different genetic makeup from that of the *Astragalus* species [14].

DNA fingerprinting can provide a definite answer to the botanical identity of the herbal medicines.

5.2 Verification of herbal formulae

The purpose of optimizing herbal formula is to simplify the herbal formulation, maximize the efficacy, and minimize the adverse reaction. A single herb contains a variety of components. But only one or a few of the ingredients may play a role in the herbal formula, other ingredients may be null and void elements that may interfere or antagonize with other active ingredients. Optimizing the herbal formula can overcome these shortcomings. Foe example in a study on diabetes mellitus foot ulcer formula, the original formula contained 12 herbs. After a series of studies, the final formula was optimized to only 2 herbs that maintain the same effectiveness of the original formula (24, 24). The volume of drug was reduced but the efficacy was improved. The effects of active ingredients were maximized and the raw herbs were saved.

Optimization of the extraction conditions and the proportion of Radix Astragali and Radix Angelicae Sinensis for Danggui Buxue Tang (DBT)

Preparation of Chinese herbal medicine in selecting suitable conditions and methods to maximize the extraction of the active ingredient is very important. In the preparation of *Danggui Buxue Tang* (DBT), an orthogonal array method (正交试验设) with three parameters of extraction, including extraction time, extraction volume and

number of repeats of the extraction was used. Traditionally, water extraction by boiling has been used for preparing DBT; however, the optimized conditions of extraction have not yet been determined. To find out the optimized extraction condition, the RA-dervied *astragaloside* IV, *calycosin* and *formononetin*, and RAS-derived *ferulic acid* and *ligustilide* in the DBT were quantified in according to the orthogonal array design. The biological activities were evaluated, which included enhancement of proliferation and differentiation on cultured MG-63 cells, estrogenic effect on cultured MCF-7 cells and anti-platelet aggregation property. The optimized conditions for extraction were therefore established (27).

The chemical and biological properties of DBT, prepared from different ratios of the drugs, were determined. Significantly, higher amounts of Radix Astragali-derived astragaloside IV, calycosin, and formononetin and Radix Angelicae Sinensis-derived ferulic acid were found in DBT with Radix Astragali and Radix Angelicae Sinensis in a 5:1 ratio. The levels of active constituents and biological activities of DBT extracts were investigated with preparations of RA and RAS at ratios of 1:1, 2:1, 3:1, 4:1, 5:1, 7:1 and 10:1, the drug ratio of 5:1 produced the best effects (26, 27).

From the angle of chemicals to evaluate the quality

Li Dongyuan (李東垣 1247 AD) documented that RA and RAS combined at a ratio of 5:1 demonstrated the best efficacy. In a previous study (26, 27), DBT was prepared by boiling the herbal mixture under various conditions and the results indicated that the 5:1 ratio indeed provided the maximum levels of active constituents of DBT.

Used as chemical markers, the main active constituents in DBT include RA-derived astragaloside IV, calycosin and formononetin, RAS-derived ferulic acid and ligustilide, and total saponins, total flavonoids and total polysaccharides (26). The detected levels of the chemical markers varied significantly among the above-mentioned seven

preparations. The level of astragaloside IV of the 5:1 ratio preparation was the highest, 2-fold higher than the 10:1 ratio preparation that recorded the lowest level (27). The 5:1 ratio preparation also contained the highest level of calycosin, formononetin, and ferulic acid. As regards the levels of total saponins, total flavonoids and total polysaccharides, the 5:1 ratio DBT preparation showed the highest levels (27).

5.3 Uncertainty of Herbal Supply—implementation of Good Agriculture Practice (GAP)

Quality control of herbal medication starts at the level of the starting material. The plant material is the most important factor in manufacturing herbal medicinal products. Herbs are inevitably "irregular" because their composition may be influenced by multiple factors, such as origin, growth, harvesting, drying, transportation and storage conditions. Through the use of cultivated herbs, some causes of variability may be eliminated. During the courses of cultivation and processing, the GAP requirements are very important in maintaining the consistency of quality.

Quality, efficacy and safety are parameters that are required for all medicines as well as Chinese herbal medicine. Consistent and reproducible quality of herbal raw materials used for medicinal purposes is paramount for clinical efficacy, and for the reproducibility of beneficial effects as observed in clinical studies. Furthermore, numerous safety issues are dependant on consistent composition of herbal ingredients. Theoretically, raw herbal material for the production of herbal medicines should come from a traceable and reproducible source. In reality, the trading habits and the growth of the market frequently obscure the origin of the herbal material and facilitate adulterations, which have already damaged the reputation of herbs.

In the Chinese Medicinal Herbal market, the quality of the supply is hinged closely with the original habitat of the herb. It might appear just a simple practice of planting the herbs in the traditional area of production, so that the best quality supply of herbs could be maintained. However, the issue is much more complicated. An herb might appear identical to what is described in the Chinese Materia Medica or in Botanical classics, and yet the chemical and biological nature expressed in its extract might not reach the desired quality. The traditional uses of herbs rely on quality suppliers which ensure quality origins, i.e., *Daodi* (道地) origin (28). Modern technology has revealed that the quality of *Daodi* should be related to special chemical and biology profiles expressed in DNA patterns, which could be identified with chemical and biological tests. With full recognition of the modern interpretation of the *Daodi* supply of herbs, GAP aims at the production of herbs, not only of identical morphological characteristics, but of ideal chemical and biological profiles. Planning for GAP, therefore, is not only confined to the place of cultivation, but involves the genetic conditions of the seeds, the field environment, the growing and harvest procedures, storage etc. (29)

The complex requirements for GAP have arisen from a more complex need apart from that of a quality supply and a good sale for the supplier. The supply of herbs with detailed qualities has become a practical issue because of the multiple needs of safety, effectiveness and research uniformity. Indeed the assumption that natural healing causes no harm should be challenged. Adverse effects could occur as a result of an inaccurate supply of herbs, adulteration from either the supplier or prescriber, or problems arising from the consumer himself. Interactions with other drugs being consumed might be the other reasons behind adverse effects (30). Situations of such nature are happening more and more frequently, creating professional problems or

sometimes, even legal challenges. To avoid such mishappening, GAP measures could greatly help (31). On the research side, the need for a quality supply of herbs is of vital importance. Until the exact active components with their chemical characteristics are known, or until its pharmacological action is known, using a herb or a combination of herbs would need a good guarantee for its safety and quality. The availability of a quality supply from a GAP will not totally relieve of the concerns about safety and quality, but will significantly simplify the complexity of the procedures required for the related authentication. The need for GAP, is therefore of multiple purposes, not only with regard to the practice standards, but also to the research requirements in a basic demand for uniformity and repeatability. GAP could be considered as an essential step towards the modernization of Chinese Medicine.

In 1998 the concept of GAP was first put forward in Chinese Herbal Medicine, and the GAP work began to be carried out since 2001 after several times of discussion during 1998-2000 organized by the State Administration of Traditional Chinese Medicine of China (SATCM), State Food and Drug Administration of China (SFDA), and China National Group Corp. of Traditional & Herbal Medicine (32, 33). A summary of the recommendations is given in **Table 5-3**.

Table 5-3 The main GAP principles for the cultivation of medicinal plants (34)

Chapters	Items	The main contents
Chap 1	General principles	Purpose and significance
Chap 2	The environment of the cultivation area	The detail request for the ecological environment such as air, water, and soil conditions in the cultivation area
Chap 3	The germplasm and breeding material	The plant or animal species should be identified correctly and the quality of the germaplasm resource should be controlled.
Chap 4	The management of cultivation	The cultivation process, such as how to use fertilizer, soil, water and how to control the insect pest and plant diseases, should be controlled by SOP (standard operating procedure) principles.
Chap 5	The harvest and process at the harvest place	The optimal harvest time should be studied and fixed. The specific request for process, drying conditions, etc. is clearly written in this chapter.
Chap 6	Package, transport and storage	It should be clearly recorded for each batch of the drug materials. The request for the transport, such as using clean container, for the storage, such as light, temperature, and humidity, is clearly provided in this chapter.
Chap 7	Quality control	The specific request for quality control, such as the items to be checked, the request for the characteristic, foreign matter, water, and ash content, is clearly provided in this chapter.
Chap 8	The equipment and operator	This chapter provides the request for the trained operators, the request about the product and process place, and equipment.
Chap 9	The document management	It should be recorded in every detail and particular for the whole process of cultivation, process, transport and storage, etc. The document should be kept properly at least five years.
Chap 10	Supplement	Supplementary explanation

Beginning with the variety of the plant, the seeds, a suitable cultivation, the time of harvest, the preparation by drying and freezing, respectively, the milling and storage are responsible for the quality of the herbal drug material (35).

The quality of an herbal medicine is defined by the quality of the raw herbs; the manufacturing of the drug preparations and the properties of the finished product is vastly diversified. Trying to narrow down the scope, the minimal combination of herbs for a formula needs to be worked out. Simple herbal formula does not mean less effectiveness, however it may facilitate the quality control. The quality control of the complete process is based on pharmacognostic methods, characteristic fingerprint chromatograms, defined amounts of marker substances, physicochemical characteristics and microbiological monitoring. Working with natural products is different from pure compound drugs. Pharmaceutical firms wish to identify the single component for a particular natural product. It remains clear from the TCM repertoire of experience that the different combinations inside one herb can produce different

useful effects on the person taking them.

5.4 Chemical markers and standard materials

Drugs as a special commodity for disease prevention and treatment, must meet the basic requirements of "safety, efficacy, controllability and stability". In order to realize the modernization and internationalization of Chinese medicine, Chinese herbs, extract, granules, prepared Chinese medicine (中成药) as the main forms of clinical medication, must be consistent with above mentioned requirements. However, the unique characteristics of Chinese herbal medicine is reflected in the diversity and complexity in composition, instability of quality, ambiguity of mechanism, and the difficulty in the control of production process, etc. To ensure the accuracy of clinical efficacy, it is necessary to ensure drug quality between batches in the stability and repeatability of production. Chemical markers and standard raw herbal materials are important basis to guarantee the quality of Chinese herbal medicine.

Chemical marker is standard substance used to test (qualitative or quantitative) the quality of study herbal drug. It can be used to determine the authenticity of drugs and drug quality to ensure the safety and efficacy. Chemical marker of Traditional Chinese medicine is the specific concept derived from the chemical marker of Western medicine. Chemical marker of Traditional Chinese medicine is an indispensable element in quality control.

Chemical markers are divided into three levels:

1) International standard chemical markers: The chemical markers are prepared, calibrated and distributed by the International Chemical Marker Center of World Health Organization (WHO)

2) National standard chemical markers: The chemical markers are prepared,

calibrated and distributed by National Institute for the Control of Pharmaceutical & Biological Products (NICPBP, 国家药品生物制品检定所)

3) Operational standard chemical markers: Prepared by the relevant units / institutes according to the operational needs

However, chemical markers of Traditional Chinese Medicine only possess national standard and operational standard but not international standard. Currently the supply of chemical markers of traditional Chinese medicine in China is very insufficient. The state has announced that there are more than 900 chemical markers of traditional Chinese medicine, however, NICPBP can only provide less than 400 of them (36). It seriously affected the quality control and clinical efficacy study of traditional Chinese medicine.

Why TCM or natural medicine is clinically effective, there must be a material basis for its activity. The research for material basis of traditional Chinese medicine is fundamental in quality and efficacy. Chemical markers provide assurance in the quality control of traditional Chinese medicine. However, due to long term inadequate attention in basic research on chemical markers of traditional Chinese medicine the active ingredients of many Chinese herbal medicine are still unknown, and this condition affects the quality control and efficacy study of TCM.

Standardization refers to measures taken to ensure that there is a consistent quantity of a defined marker compound within an herbal material, as herbal materials are known to be highly variable in their make-up (37). Intrinsic factors (e.g. genetics) and extrinsic factors (e.g. growing, harvesting, storage and drying processes) may lead to variations in the chemical profiles of the herb (38, 39).

In order to achieve reproducible biological data in terms of safety and efficacy, it is

important that the herbal material should be standardized to the active ingredients when they are known or to specific markers when the active ingredients are not yet known (40). Chemical fingerprints are commonly used to confirm the identity, authenticity and lot-lot consistency of a plant (41).

An herbal product cannot be considered scientifically valid if the herbal product was not authenticated and tested to ensure reproducibility in the manufacturing. Many studies refer to the use of standardized material, but in reality they are referring to chemical standardization.

Each of the principal herbs should be standardized as to the content of the major active compounds (many of which might be unknown). The objective is to establish a chemical "fingerprint" that meets certain standards for each lot of a particular herb. The actual formulation always will be based on a mixture of such standardized herbs.

Chemical markers which are used for drug quality control are divided into chemical drug markers and Chinese herbal drug markers. Chemical drug marker is often refined from raw material of the chemical drug that is easily available and inexpensive. Chinese herbal drug markers are hard to get and the price is expensive because of the constrained raw herbal material, and the complex process of isolation and identification. But it is very important and can not be avoided in research and quality control of traditional Chinese medicine.

As a standardized substance, there are specific criteria and requirements.

1) Purity: Chemical markers used for content determination the purity should be over 97%;

2) Stability: It should be thermally and lightly stable. For example, Tanshinone Ⅱ A solution, at room temperature the content is continuously reduced with the increase in

the number of placement days;

3) Uniformity: In the preparation of herbal markers, repeated crystallization often happen, which tends to make the solution uneven. So, it is necessary to mix the marker evenly before use (42).

The overall quality of an herbal medicine may be affected by many factors, including seasonal changes, harvesting time, cultivation sites, post-harvesting processing, adulterants or substitutes of raw materials, and procedures in extraction and preparation. From harvesting to finished product, chemical markers play a crucial role in evaluating the quality of herbal medicines. However, at present, some herbs do not have markers for quality control. According to the Chinese Pharmacopoeia (2005 edition), only 281 out of 551 herbs have one or two chemical markers for quality control.

Apart from quality control, chemical markers are applicable to many research areas, including authentication of genuine species, search for new resources or substitutes of raw materials, optimization of extraction and purification methods, structure elucidation and purity determination. Systematic investigations using chemical markers may lead to discoveries and development of new drugs.

In the DBT project, *Radix Astragali* is graded according to its diameter, length and physical appearance. *Isoflavonoids* and *saponins* were recognised as the major bioactive components attributed to the therapeutic effects. These two types of components were used to evaluate the quality of *Radix Astragali*.

Determination of active ingredient is an important criterion for quality control.

However, some contents are not really active ingredients, but landmark composition, representative ingredients or principal components; some are one or two ingredients of a variety of active ingredients. It is insufficient to control the quality of herbs only based on the few markers. Some markers are only for measurement of the active ingredient, and not references for toxic and hazardous components testing. More important, a large number of unknown elements are completely out of control. Many unknown ingredients that may be active ingredients or toxic substances, are closely related to the drug's safety and effectiveness, but can not be effectively controlled.

Uniformed, normed, and stabilized quality standard is very important. But for many Chinese herbal medicines, the quality standards are not unified, non-standardized, and precarious. For example, the same drug in different dosage forms; or the same drug and the same dosage form from different manufacturers; even in the same drug, the same dosage form, and the same manufacturer in different batches, all of these make the quality variable, the quality standards confused.

Quality control can be a multi-disciplinary approach, so far only chemical marker is workable and practical.

In addition to chemical marker and DNA fingerprint, to limit the herbal composition in a formula may make it easier to achieve quality control. Under the precondition of keeping effectiveness unchanged, optimizing the complex herbal formulae is another way to improve quality control. For example, the herbal formula of Diabetic Mellitus Foot Ulcer (DMF) the original formula had 12 herbs, after optimizing the formula contains only two herbs, which greatly simplify the quality control.

5.5 Manufacturing trial agent—Good Manufacture Practice (GMP)

The quality of a TCM medication is defined by the quality of the herbal drug, the manufacturing of the drug preparations and the properties of the finished product, taking into account the special requirements of the individual herbal species in accordance with Good Manufacturing Practice (GMP) standards. The quality control of the complete process is based on pharmacognostic methods, characteristic fingerprint chromatograms, defined amounts of marker substances, physicochemical characteristics and microbiological monitoring.

Good Manufacturing Practice (GMP) is a component of quality assurance that ensures a pharmaceutical product manufactured with a quality appropriate for its intended use on a consistent basis [43].

We take the *Danggui* Extract (當歸提取物顆粒劑) as example to elucidate the quality control and GMP implementation.

The compositions of the product

(1) Water extract of Danggui;

(2) Lactose.

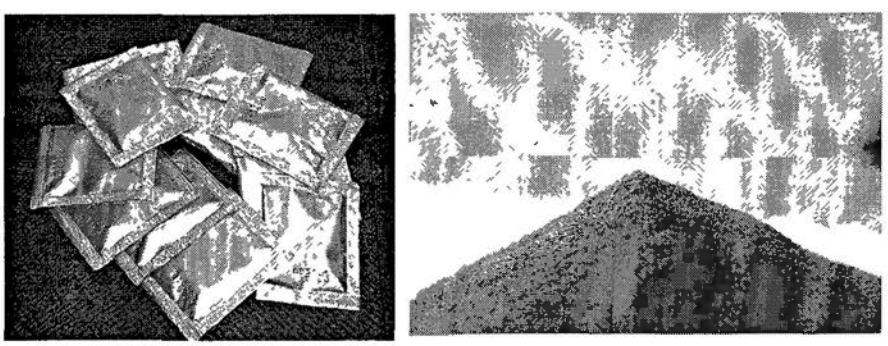


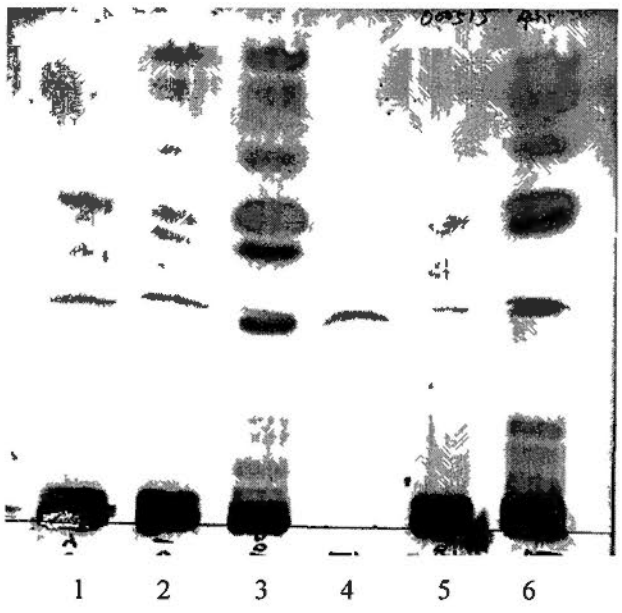
Figure 5-2 Danggui Extract and Sachet

Dosage form specifications:

Visual appearance	*Homogeneous in brown and fine granule, no agglomerate*
Odor and taste	*Spicy characteristic of Danggui, slightly sweet in taste*
Solubility	*Soluble in warmhot water*
Moisture content	*<5%*
Package (grams)	5
Uniformity of package	*<10%*

Chemical component specifications (please refer to the analysis results as attached)

Marker detection	*Ferulic acid*
Identification	*Comparable to the crude herb on TLC*
Quantitative control	*The concentration of Ferulic acid should not less than 100 μg/gm on HPLC*



1 Danggui Extract Solution, E-DG002(WO)
2 Danggui Extract Sachet, S-DG001 (WO)
3 Danggui herb, H-190100(WO)
4 Ferulic acid, Standard
5 Danggui tablet, T-DGBX001(MHS)
6 Danggui herb, H-261099(MHS)

Figure 5-3 Thin Layer Chromatogram (TLC) Analysis for Danggui Samples

Conditions:

Plate: Silica F254 pre-coated

Solvent System: Benzene/Chloroform/Methanol (2:2:2:1, v:v:v)

Spray Reagent: Ethanolic 10 % H_2SO_4

Coloration: Store in 105°C oven for 5 mins

Sample preparation: Samples were cut and ground into powder and sonicated with methanol / glacial acetic acid (95:5, v:v) for 1 hour. Then

sample extracts were filtered through 0.2 μm Nylon membrane for TLC and HPLC analysis.

Conclusions:

Based on the TLC chromatograph of the product, the results indicated that the extract retained the maker component (ferulic acid) as well as other components:

- Comparable bands with the crude herb;
- The maker component, Ferulic acid.

Table 5-4 High Performance Liquid Chromatogram (HPLC) Analysis of Ferulic acid in *Danggui*

Sample & ID	Concentration (μg/mL)	Ave. Concentration (μg/mL)	Concentration (μg/30 mL)	Weight (gm)	Corrected Concentration (μg/gm)
Danggui herb (WO) H-190100	21.25 21.66	21.46	643.8	5.000	128.8
Danggui herb(MHS) H-261099	35.24 35.33	35.29	1058.7	5.000	211.7
Danggui Extract Sachet (WO), S-DG001	94.77	95.35	2860.5	4.279	668.5

Sample preparation: Samples were cut and ground into powder and sonicated with methanol / glacial acetic acid (95:5, v:v) for 1 hour. Then sample extracts were filtered through 0.2 μm Nylon membrane for TLC and HPLC analysis.

Conclusion:

The ferulic acid concentration in the product was 668.5 μg/gm (>100μg/gm, as the chemical component specifications).

The manufacturing procedures:

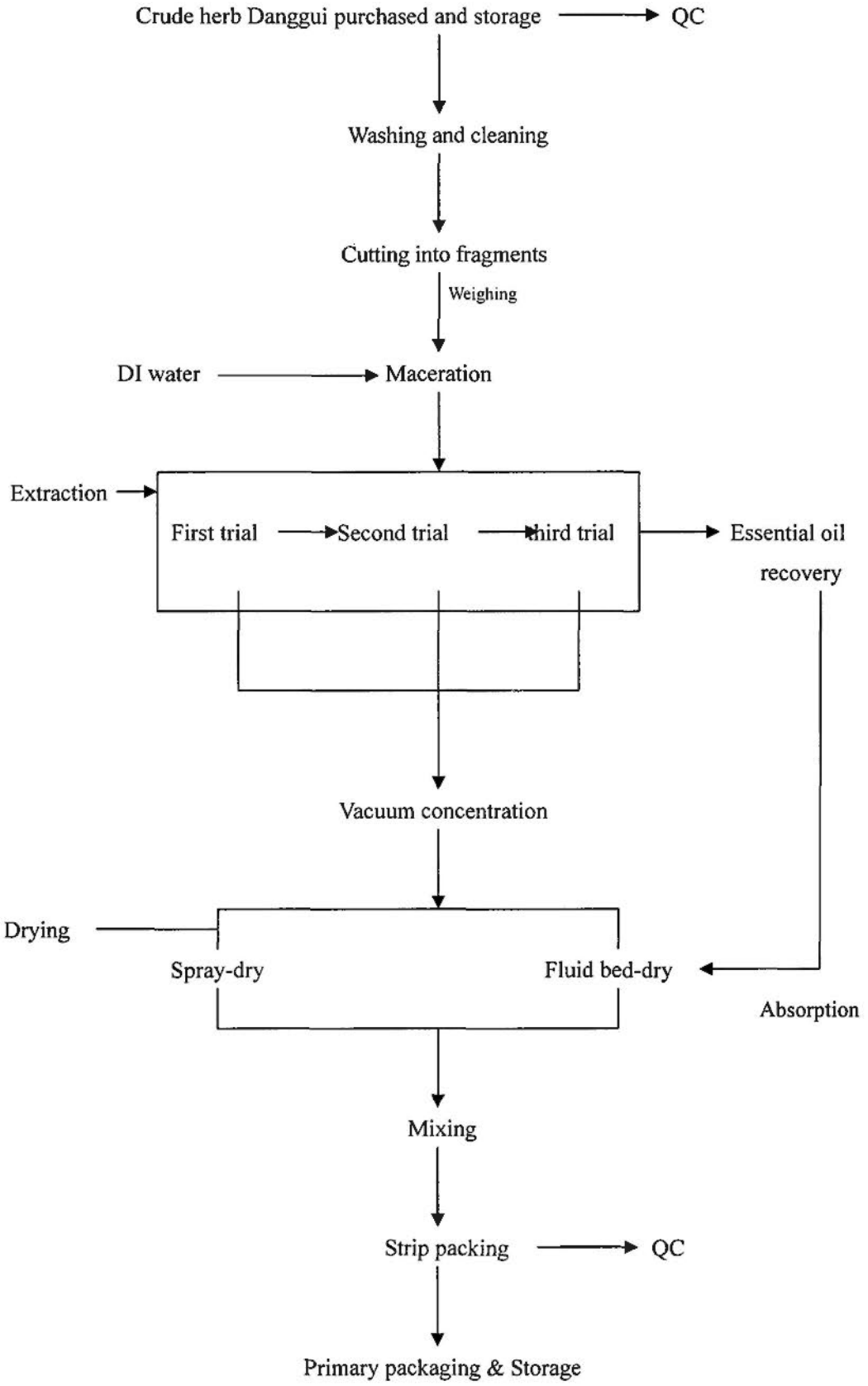


Figure 5-4 *Danggui* Extract Sachet manufacturing flow chart

Table 5-5 Quality monitoring of crude *Danggui* root

Origin of plant	Radix Angelicae sinensis 伞形科植物當歸 Angelica sinensis (Oiv) Diels 的乾燥根
Distribution	Ganshu 甘肅
Lot number	WO070300
Visual appearances 	The main root is short, with longer branches from its extremity which are more or less the same length. The entire root is about 15-25 cm long, and 3-3.5 cm diameter at the head. The brown longitudinally grooved, often thin, pieces of root have radially arranged canals (100-200 um) in the bark and a radially striated yellow xylem. In addition, there are irregular fragments of rhizome, likewise with secretary canals.
Odor & taste	Characteristic smell of Danggui, slightly sweet taste.
Fluorescence identification	The cut facets of the crud herb showed bright write fluorescence under UV365nm
Marked component identification	Ferulic acid band on TLC

Wash and clean the crude *Danggui* root

Cut the clean herb into fragments no bigger than 6 cm in a total of length, width and thickness

Extraction-Concentration process

Table 5-6 Extraction & Concentration System

Solvent and the amount used	Water, 8 times of 10kg *Danggui* to be extracted.
Maceration	16 hours at room temperature
Temperature	100 °C.
Extraction intervals	3 times
Boiling time	1 hour for the first two trials, and 0.5 hour for the third trial.
Recovery of essential oil	Collect the essential oil and mixed with distilled water
Concentration	Concentrated the extract to density of 1.08-1.10 under –660 mmHg at 60°C.

Dry the *Danggui* concentrate to powder

Spray-Dryer ----- dry the Danggui concentrate to be powder

Fluid-Bed ----- absorb the essential oil into dry powder

Mix the *Danggui* extract powder and the excipients

Sieve the mixed *Danggui* powder under 20 meshes

Sachet packaging

Primary packaging and Labeling

Packaging ----- carton of 4 vacuumed plastic bags of 100 sachet bags

Labeling ----- (see the attached sticky label as below)

Product inserts in Chinese and English

Consistency of production

Three batches of *Danggui* extract were produced and tested under the optimum conditions developed based on experimental studies.

The quality of a herbal multi-component product cannot only be defined by its release specification. The complete production process is a precondition for a defined quality of the medicinal product, including the use of herbal drugs of defined quality, the manufacturing of the extracts and the finished product in concordance with the GMP standards (Good Manufacturing Practice), and the analytical control of all intermediate products and the finished product. Besides a well-defined process, still wide experience on the manufacturing of the extracts and the finished product is required to produce a reproducible product of high quality.

The identity and quality is secured by tests covering all components of the combination. Tests parameters include TLC fingerprint chromatograms covering all of the polarity range, the appearance (color, clearness), the density, the content of ethanol, the microbiological purity and the content of all nine components by the assay of

characteristic marker substances. The proportion of each component in the final product has to be within a range of 95–105% of the specified content. In **Fig. 5-4** the complete manufacturing and quality control scheme is shown.

The degree of quality control depends on the manufacturer, the supplier, and others in the production process. Good Manufacturing Practice (GMP) is a component of quality assurance that ensures a pharmaceutical product manufactured with a quality appropriate for its intended use on a consistent basis [44]. In practice, this means that the manufacturing process is completely defined, both in terms of materials and procedures, from start to finish.

There are several challenges that need to be addressed when applying the principles of GMP to plant-derived pharmaceuticals. The concept of pharmaceutical GMP was originally developed for drugs manufactured by chemical synthesis and later adapted for biopharmaceuticals produced by cell culture and fermentation technologies in closed, precisely monitored and controlled systems. Moving on from the cultivation and harvesting of plant material, GMP must also be applied to the subsequent downstream processing stages of pharmaceutical production. This involves extracting the pharmaceutical product from harvested plants. The initial stages of processing (harvesting, extraction, clarification) are very changeable and have to be optimized in a system-specific manner.

Chapter 6

Overcoming the Difficulties---Safety and Efficacy Evaluation

Traditional Chinese medicine (TCM) has been an experience-based practice over the past five thousand years. The safety and efficacy of TCM were proved through experience, rather than present modern scientific assessment. The criteria for efficacy and safety of Chinese herbal medicines should be the same as those for chemical drugs. Many Chinese herbal medicines have a long history of traditional use. However, most are of unproven efficacy by today's standard, i.e. well-designed randomized controlled trials and comprehensive pre-clinical studies. Although the lack of evidence does not mean that Chinese herbal medicines lack efficacy or are unsafe, rigorous designed experimental and clinical investigations should still be done and more suitable research methods for Traditional Chinese medicine should be sought for. So far approaches to the study of herbal drugs are still using the research methods for chemical drugs.

6.1 Pre-clinical efficacy evaluation

For Chinese herbal medicine, there is a need to scientifically proof and clinically validate its safety and efficacy through chemical standardization, biological assays, animal models and clinical trials. Quality assurance of herbal preparations is the prerequisite of credible clinical trials.

6.1.1 Pharmacological study

Pharmacological studies of Chinese medicine are much more complex when compared with chemical drugs. First of all, we do not exactly know what really works inside the composition of traditional Chinese medicine and it means we do not exactly know the active ingredients in the herbal formula. The marker compounds we used for quality control and pharmacological study may not be the active ingredient, or may be just part of the active ingredient. Pharmacological studies carried out under such conditions have many uncertainties, and therefore the comprehensiveness and accuracy can be imagined; this is a major obstacle to traditional Chinese medicine research. However, pharmacological research on Chinese medicine cannot stop because of the obstacle. We still need to use currently available knowledge and skills to explore the mechanism and modalities of Traditional Chinese Medicine. Two of the important research methods are *in vitro* and *in vivo* studies.

in vitro efficacy study

In vitro study is performed not in a living organism but in a controlled environment. It is better suited than *in vivo* research for deducing biological mechanisms of action. For example in DBT project, according to TCM theories the herbal medication is able to replenish *qi* and nourish *xue* (the blood) (1). Menopausal women are often suffering from symptoms of *qi* and blood deficiency; therefore it is used for treating menopausal symptoms. Due to deficiency of ovarian hormones, especially estrogen, women in menopause often suffer from hot flashes, sweating, anxiety, mood swings and an increased risk for other health problems, such as reduction of bone mineral density and cardiovascular diseases (2).

In the *in vitro* studies, various bioactivities related to menopausal symptoms, such as osteotropic effect, estrogenic effect, anti-platelet aggregation effect and

immunomodulatory effect have been used to evaluate the functional roles of DBT.

DBT extract was applied to cultured human MG-63 osteosarcoma cell. Bone cell proliferation and differentiation were measured by 3-(4, 5-dimethylthioazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay and alkaline phosphatase (ALP) assay. DBT can induce both the proliferation and differentiation of osteoblast MG-63 cells in a dose-dependent manner. In both assays, DBT showed stronger effects than RA or RAS alone. In the MTT assay, the 5:1 ratio DBT extract stimulated MG-63 cell proliferation, which was 10–20% higher than the extracts of other ratios (**Figure 7-1**). For bone cell differentiation, the 5:1 ratio DBT preparation induced ALP activity to the highest level among all ratios and showed the strongest osteotropic effect (3).

The estrogenic effects of DBT were also tested by a cellular reporter system of transcriptional activation of estrogen receptor/promoter. A promoter/reporter construct corresponding to the responsive elements of estrogen receptor was stably transfected into MCF-7 cells. The DBT extracts of various ratios were applied onto the cultures for 2 days. Two parameters, namely cell number and promoter activity (luciferase activity), were determined. While DBT was not able to alter the proliferation of MCF-7 cells, the estrogen-driven promoter activity was markedly induced by DBT (**Figure 6-1**); the 5:1 ratio DBT showed the strongest effect in inducing the promoter activity than RA, RAS alone or the extracts of other ratios [3].

In anti-platelet aggregation assay, the activity of DBT in preventing ADP-induced platelet aggregation was determined. The ratios 5:1 and 7:1 DBT extracts demonstrated higher levels of activity in preventing platelet aggregation than either RA, RAS alone or the extracts of other ratios (**Figure 6-1**) (3).

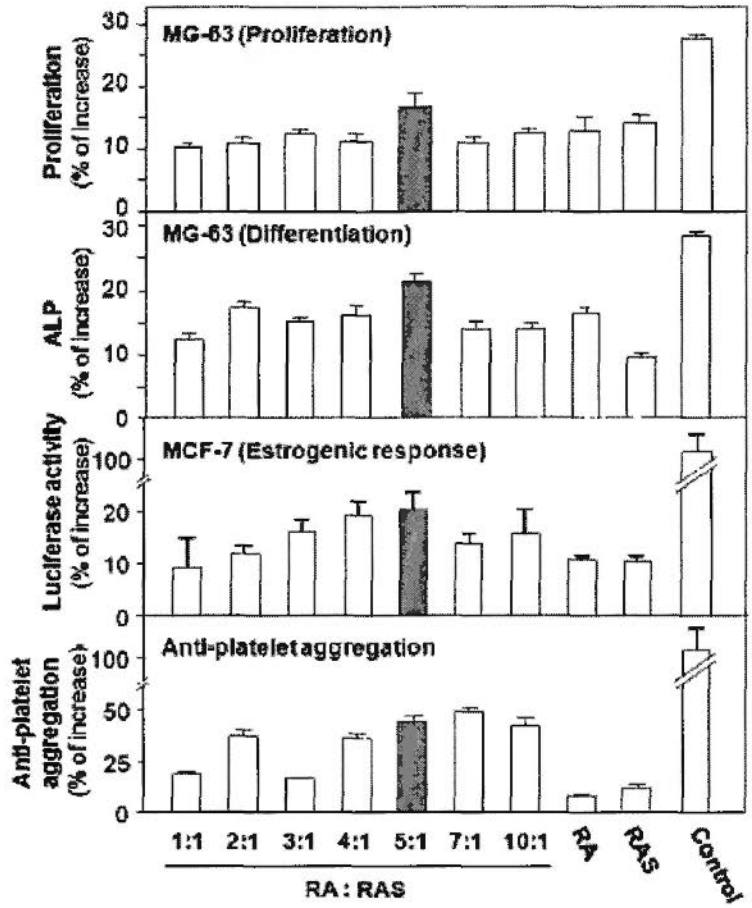


Figure 6-1 Biological activities of RA, RAS and DBT of various RA to RAS ratios.

In a study of immuno-modulatory effects, DBT preparations of various ratios were applied to cultured T-lymphocytes and macrophages. In cultured T-lymphocytes, DBT induced marked cell proliferation, interleukin-2 secretion and the phosphorylation of extracellular signalregulated kinase (ERK1/2). In addition, the phagocytosis of cultured macrophages was elevated by DBT treatment. The immuno-modulatory effects of 5:1 ratio DBT were the strongest (4) (**Table 6-1**).

Table 6-1 Biological evaluation of DBT (in vitro studies)

Findings	Model	Treatment	References
The 5:1 ratio DBT showed stronger effects in	Cultured human MG-63	DBT of various ratios of RA and RAS, compared with	Dong et al. [3]

stimulating MG-63 cell proliferation and induced ALP activity to the highest level among all groups.	osteosarcoma cells	β-estradiol and negative control	
The 5:1 ratio DBT showed the strongest effect in inducing the estrogen-driven promoter activity than RA, RAS alone or the extracts of other ratios.	Cultured MCF-7 cells	DBT of various ratios of RA and RAS, compared with β-estradiol and negative control	Dong et al. [3]
The ratios 5:1 and 7:1 DBT showed higher levels of activity in preventing platelet aggregation.	ADP induced-platelet aggregation in blood from adult New Zealand white rabbits	DBT of various ratios of RA and RAS, compared with ticlopidine and negative control	Dong et al. [3]
DBT induced cell proliferation, interleukin-2 secretion and the phosphorylation of extracellular signal-regulated kinase (ERK1/2) in cultured T-lymphocytes. The 5:1 ratio DBT showed the strongest immuno-modulatory effects.	Cultured T-lymphocytes and macrophages	DBT of various ratios of RA and RAS, compared with PHA, PMA, Zymosan A and negative control	Gao et al. [4]

In addition to the in vitro assays, the 5:1 ratio of RA and RAS in DBT was further tested and verified by animal studies. In DBT-administrated mice, the 5:1 ratio preparation was the most effective decoction in triggering immune responses (5, 6).

Serum pharmacology of Traditional Chinese Medicine

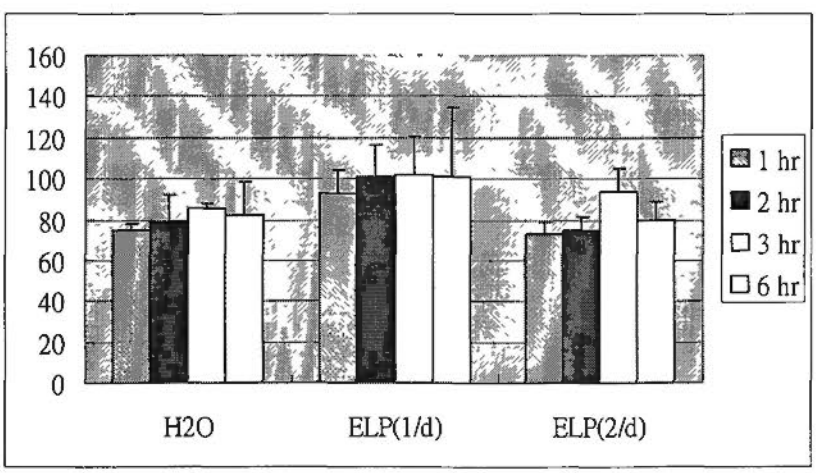
Serum pharmacology of Traditional Chinese medicine was first put forward in 1987 by Japanese scholar Hiroko Iwama (7). The methods of Serum pharmacology of Chinese medicine include orally administrating study herbal drug, collecting blood sample after a certain time, and separating serum. This method is an experimental technique by using the active ingredient contained serum to conduct in vitro pharmacological study. It is a scientific method that is more suitable for TCM research. Serum pharmacology of Chinese medicine not only lay the foundation in the

study of variety of active ingredients, but also in the pharmacokinetics of Chinese herbal formula, Eventually, it will provide a credible scientific evidence for clinical application of Chinese herbal formula and its dosage modification.

The one of the most important advantages of Serum pharmacology is reproduced *in vivo* environment through in vitro experimental system. In vitro experimental results obtained from serum pharmacology are consistent with in vivo results. It not only can reflect the direct effect of absorbable part of the drugs, but also reflect the indirect effect induced by the endogenous drug metabolites in the body (8).

Evaluation of TCM using serum pharmacological methods is direct, rapid, sensitive and accurate, which can be widely used in the evaluation of herbal compound.

Concentration of active ingredient of Chinese medicine in serum is affected by many factors such as dosage (concentration of drug), feeding frequency, time for blood collection and duration of feeding. In our 壯骨關節膠囊 (ELP) study, to obtain maximal concentration of active ingredients in serum, we tried different dose, different blood collection time and different feeding duration. We found that 4g 壯骨關節膠囊 (ELP) once a day and 2-3 hours post-treatment showed higher level of ALP activity (**Figure 6-2, 6-3**). (9)



H2O: *Feeding with 4ml distilled water*
ELP (1/d): *Feeding with 4g ELP once/day*
ELP (2/d): *Feeding with 2g ELP twice/day*

Figure 6-2 ALP Activity in different dose and time of blood collection

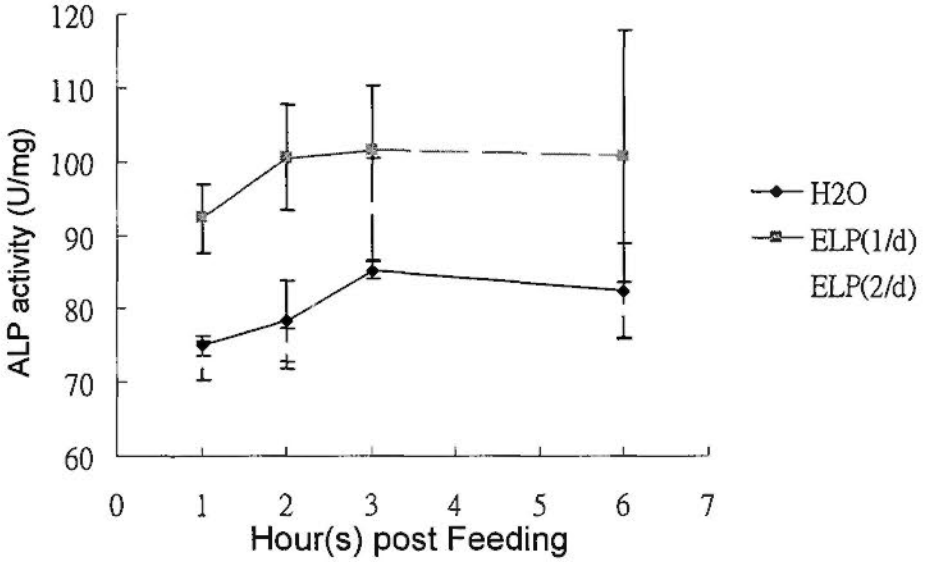


Figure 6-3 ALP activity in different post-treatment time

6.1.2 Animal models in TCM research (*in vivo* study)

The advantages of *in vitro* study are short test cycle, easy operation and low cost. As the testing materials have not been metabolized in the body but directly in contact with target cells, the results cannot really reflect the actual situation. It is only worthy as a reference or for screening purposes. Although *in vivo* study needs longer research duration with complicated operation and high cost, the testing materials are metabolized through body's metabolic process and then contact with the corresponding target cells, it is hence closer to the real situation. It is an essential part of drug research.

in vivo efficacy study of DBT

The pharmacological studies in animals suggest that DBT has the ability to promote hematopoiesis, to stimulate blood circulation, to prevent osteoporosis and to counter

oxidative stress (3, 10, 11). DBT is known to enhance myocardial mitochondria and glutathione status in red blood cells, thereby increasing their resistance to injury induced by oxidative stress (12). In rats, DBT protected against myocardial ischemia-reperfusion injury in a dose-dependent manner (12). A more potent cardio-protection was demonstrated in DBT-treated rats than in rats treated with either extracts of RA, RAS alone, or a mixture of RA and RAS (not boiled together).

When the mice were administered orally with DBT, the serum collected from abdominal aorta was added to an in vitro cultivating system of mouse hematopoietic progenitor cells. The decoction-contained serum showed promoting actions to CFU-GM and CFU-E. Once again, the action of the 5:1 ratio DBT was 97.81% stronger than that of the 1:1 ratio extract (13, 14) (**Table 6-2**).

Table 6-2 Biological evaluation of DBT (in vivo studies) (15)

Findings	Model	Treatment	Reference
DBT had significantly higher RBC and Hb levels in both normal and anemic mice than those in RA, RAS and control.	Kunming mice, male, RBC, Hb	Normal mice in 4 groups: RA, RAS, DBT and control; Anemic mice in 4 groups: RA, RAS, DBT and control	Wu BC et al. [5]
DBT was the most effective decoction in triggering immune responses.	Kunming mice, RBC, Hb, WBC, Plt, reticulocyte, nucleated cells of bone cavity, weight of pancreas and thymus	Mice in 5 groups: RA, RAS, DBT, RA+RAS (1:1) and control	Li YK et al. [6]
DBT alleviated cardiac injury in ischemia reperfusion.	Wister rats (male), amplitudes of LVSP and ±dp/dtmax, arterial pressure, Na+-K+-ATP activity, level of MDA production, cAMP content	Rats in myocardial ischemia reperfusion injury; i.v.	Wu DZ et al. (10)
DBT increased the levels of RBC, WBC, and BMNC. Some DBT promoted the proliferation of BMNC and increased the level of CFU-Mix.	Kunming mice, ICR mice, Balb/ c mice, RBC, WBC, reticulocytes and BMNC	Mice in 4 groups: normal, model, DBT without polysaccharides, DBT with polysaccharides	Ning L et al. (11)
DBT enhanced myocardial mitochondria and red blood cell glutathione status.	Rats, myocardial mitochondrial status, RBC glutathione status	Rats in 5 groups: RA, RAS, DBT, RA + RAS (not boiled together) and	Mak DH et al. (12)

		control; orally administered	
DBT inhibited growth of GM-CFU, while the decoction-containing serum promoted growth of GM-CFU.	Kunming mice, GM-CFU	DBT was administered orally; serum collected from abdominal aorta was added to an in vitro cultivating system of mouse hematopoietic progenitor cells.	Zhang YH et al. (13)
The decoction-containing serum showed promoting actions to CFU-E. RA+RAS (5:1) was 97.81% stronger than RA+RAS (1:1).	Kunming mice, CFU-E	DBT was administered orally; serum collected from abdominal aorta was added to an in vitro cultivating system of mouse hematopoietic progenitor cells.	Zhang YH et al. (14)

In vivo study of Osteoporosis

Postmenopausal osteoporosis is a major health problem in women, the understanding of which is hindered by the difficulty of studying a disease that is restricted to humans. Osteoporosis is a slowly progressive disease, necessitating a study of several years' duration to make a conclusion for the response to therapy.

Animal models provide more uniform experimental material and allow extensive testing of potential therapies. Many species of animals such as dogs, cats and sheep can be used as animal model, but the most commonly used animal model for osteoporosis studies is the rodent (16).

In order to investigate the effects and mechanism of the studied herbal formula on bone density, animal model of osteoporosis is usually needed. Sprague-Dawley (SD) strain rats are commonly used to establish animal model. During animal model preparation, in our ELP study, SD rats were randomly divided into three groups, an ovariectomized group (OVX), OVX group treated with medical herbal decoction (OVX-M), and an OVX group treated with estrogen (OVX-E).

In vivo study of Dietetic foot ulcer

The streptozotocin (STZ)-induced diabetic rat model was used since STZ has been widely used for induction of diabetes mellitus in animal experiments (17,18,19) . Neonatal non-insulin-dependent diabetes mellitus was induced by intraperitoneal administration of STZ (70 mg/kg) to Wistar neonatal rats within 5 days of age (20). At 8 weeks of age, these rats developed hyperglycemia, hypoinsulinemia and glucose intolerance, and this condition persists for 12 weeks. The adult diabetic rats were then used for the development of the ulcer model. Only those rats with severe diabetes (plasma glucose > 300 mg/dl) were selected for wound induction. The rats were anesthetized with an intraperitoneal injection of 75 mg/kg ketamine and 10 mg/kg xylazine. A rectangle was marked on the dorsal surface of the foot using a signet, and then a layer of skin in full thickness (standard area 2 × 5 mm) was removed (**Figure 6-4**). (21)

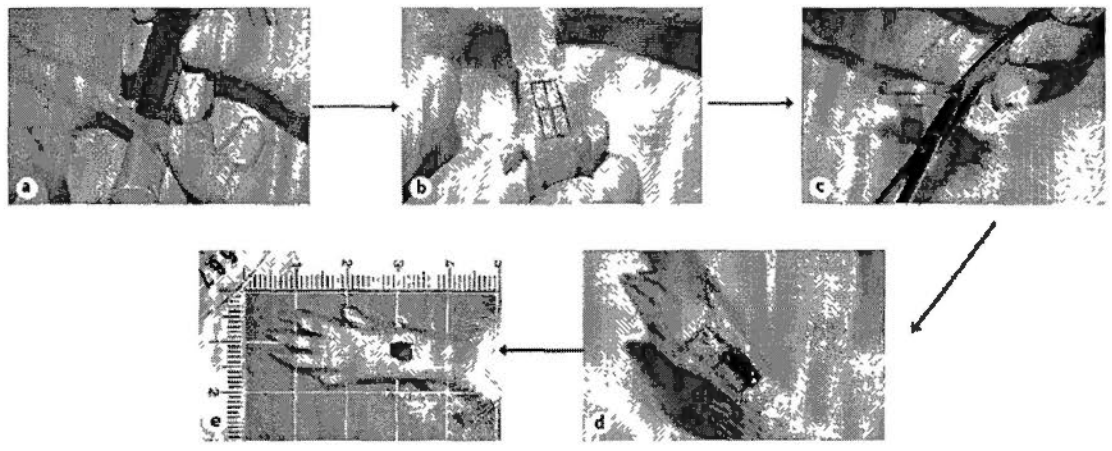


Figure 6-4 Procedures of wound induction. a. Signet marking. b. Clear boundary for wound induction. c. Removal of full-thickness skin. d. Fresh wound area of 2 × 5 mm. e. Slighty larger wound area after 1 day.

For ulcer area measurement and validation, planimetric measurements were performed on digital photographs taken from each rat's foot, and the pictures were

analyzed using the IACP.

The digital images were deconstructed into their primary components using a specifically written Pascal language image analysis computer software (Borland Delphi version 6.0; Borland, Austin, Tex., USA). The IACP employed conventional image analysis techniques such as color thresholding, edge detection and color filtering. The program also divided each image into the relevant regions of interest, namely the rulers, rat foot and wound (**Figure 6-5**).

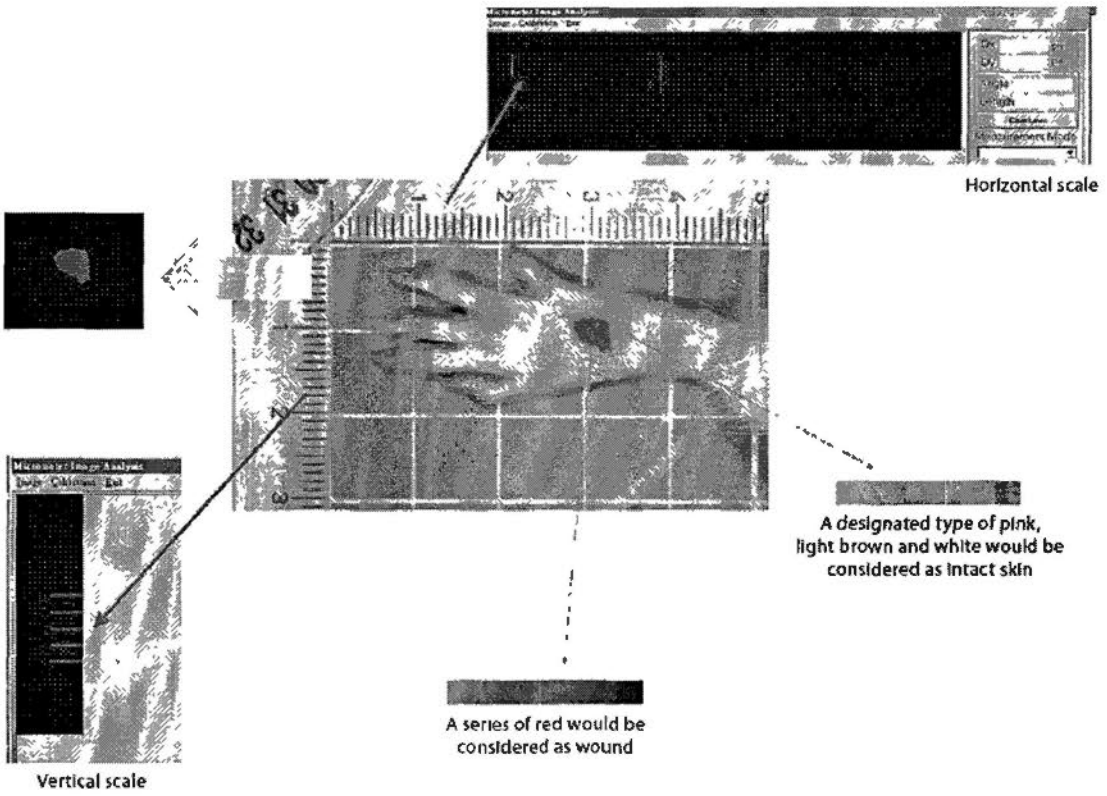


Figure 6-5 Diagram showing the procedures of wound area measurement using IACP. Horizontal and vertical charting rulers on the stand copier platform were recognized and interpreted by the IACP as horizontal scale and vertical scale. In the aspect of wound color differentiation, some template photographs with a series of red color were employed to enable the program to recognize a wound and calculate the area automatically.

In the preparatory stage of the model establishment and trial study, the wounds were

photographed on days 1, 4, 8, 13 and 18 (**Figure 6-6**).

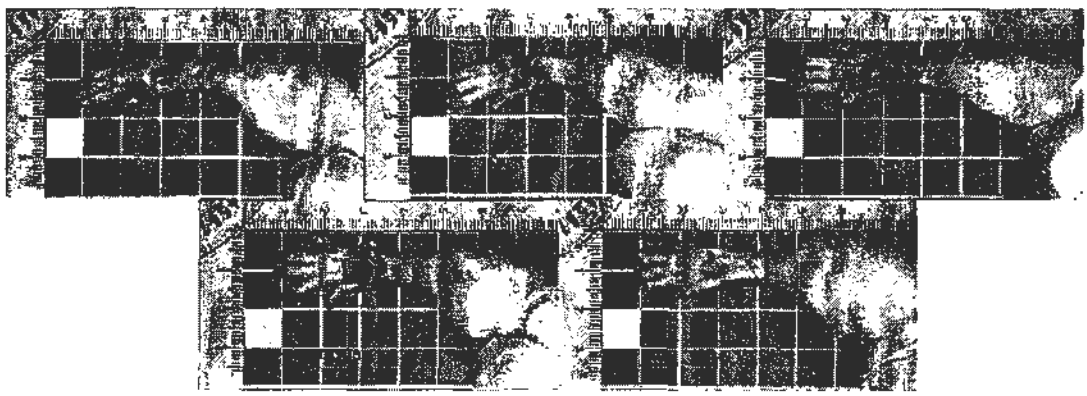


Figure 6-6 Healing progress of the wound (days 1, 4, 8, 13 and 18) in the diabetic foot ulcer animal model

Ulcers induced on feet for the study of wound healing and its measurement method has never been reported. With the aim of mimicking more closely the clinical condition of diabetic foot ulcers, the ulcers were created on the feet of STZ-diabetic rats. The animal model was probably suitable for in vivo studies of ulcers in response to different medications (21).

Postmenopausal animal model

For a long time, experimental researches have been the weak link in Chinese medicine research. This is related to the traditions of Chinese medicine. In Chapter 6 and Chapter 7 the contents have been described. Along with the development of science and technology, the methodology of traditional Chinese medicine research is also constantly developing and improving. The introduction of new technologies makes the evidence of efficacy and safety evaluation of traditional Chinese medicine more reliable. Experimental studies are generally, including pharmacology and toxicology

studies. The former focuses on the efficacy and metabolic processes of traditional Chinese medicine; the latter investigate the safety of Chinese medicine.

To further demonstrate the efficacy of DBT, we developed animal mode of climacteric symptoms to study the action mode. From a Chinese medicine perspective, menopausal symptoms are associated with a decline in Kidney *Yin* or *Yang* or a combination of both. *Dang Gui* (*Radix Angelicae Sinensis*) is one Chinese herb that is recommended for the treatment of menopausal symptoms. *Huang Qi* is used in the treatment of the menopausal symptoms to tonify *Qi*. This study is to evaluate the effect of a combination of *Dang Gui* and *Huang Qi* (DBT) on improvement of climacteric syndrome in old non-reproductive mice when administered orally for a period of 30 days.

The experiment was performed in old non-reproductive mice aged of at least 12 months. 50 study animals, which were confirmed without sexual cycle by vaginal smear examination, were selected from 100 animals.

Non-reproductive animal model which aged more than 12 months was defined as non-reproductive if 5 continuous daily vaginal smear examination confirmed without sexual cycle.

The groups, dose levels and animal numbers were shown in **Table 6-3**.

Table 6-3 Groups, dose levels and animal numbers

Group	Dose	Dose levels (g/kg /day)	Equivalent to human dose	Animal numbers
Negative control	Distilled water	0.1ml/mouse		10
Positive control	Estradiol benzoate	0.1ml/mouse		10
Low dose	DBT	0.25	5 times	10
Medium dose	DBT	0.5	10 times	10
High dose	DBT	1.5	30 times	10

The following parameters were measured:

- Serum 17 beta-estrodiol (E2): Serum was taken and E2 concentration

was measured with Photoelectric meter.

- Serum Progesterone: Photoelectric meter was used to measure Progesterone concentration.
- Uterus / body weight ratio: （Uterus wet weight/Body weight）×100%
- Uterus water uptake: ([Uterus wet weight - Uterus dry weight]/ Uterus dry weight)×100%

  The Uterus dry weight was obtained after uterus was taken and placed in a 100^0C oven for 24 hours.
- Vagina estrogen activity
- Vaginal epithelial cell Keratosis

The results indicated that Serum estrogen concentrations in high and medium dose groups were significantly higher than negative control ($p<0.05$), but Progesterone level had no change (**Table 6-4**).

Table 6-4 Changes of Serum Estrogen and Progesterone ($\overline{X} \pm SD$)

Group	No. of Animal	Estrogen pg/L	P value	Progesterone nmoL/L	P value
Negative	10	10.57±4.00	-	5.94±1.93	-
Positive	10	26.00±4.78	0.000*	24.70±13.88	0.003*
Low dose	10	13.39±2.87	0.138	8.00±2.64	0.489
Medium dose	10	14.70±5.13	0.032*	8.11±2.67	0.467
High dose	10	14.61±3.65	0.036*	8.25±3.02	0.439

Note: compared with negative control group

Uterus water uptake of three DBT treated groups was significantly stronger than control group ($p<0.01$) with a dose-dependent relationship. Although there was no significant difference in Uterus / body weight ratio between dosage groups and negative control, a dose-dependent relationship was observed (**Table 6-5**).

Table 6-5 Changed of Uterus ($\overline{X} \pm SD$)

Group	No. of Animal	Uterus wt/ Body wt ratio	P value	Uterus water uptake rate	P value
Negative	10	0.281±0.095	-	229.08±124.09	-
Positive	10	0.538±0.208	0.000	390.78±72.91	0.000*
Low dose	10	0.354±0.168	0.263	373.52±46.37	0.000*
Medium dose	10	0.362±0.164	0.171	341.90±99.64	0.001*
High dose	10	0.395±0.092	0.092	363.84±37.15	0.001*

Note: compared with negative control group

Vagina estrogen activity in high dose group was higher than negative control (p=0.027). There was no significant difference in Vaginal epithelial cell Keratosis between dosage groups and negative group, but a dose-response relationship was observed (**Table 6-6**).

Table 6-6 Changes of Vaginal epithelial cell Keratosis ($\overline{X} \pm SD$)

Group	No. of Animal	Vagina keratogenous (Positive/ Negative)	P value	Vagina estrogen activity (A value)	P value
Negative	10	2/8	-	0.13±0.03	-
Positive	10	9/1	0.005*	0.34±0.09	0.000*
Low dose	10	3/7	1.000	0.14±0.02	0.171
Medium dose	10	4/6	0.628	0.16±0.03	0.062
High dose	10	7/3	0.07	0.18±0.04	0.027*

Note: compared with negative control group

The results suggested that serum sex hormone and vagina estrogen activity and uterus water uptake were improved. A dose-dependent relationship in uterus weight was also observed. DBT may have the effects on the climacteric syndrome through elevating serum estrogen level and improving sexual organs.

DBT can improve the estrogen levels of aging mouse, increase vaginal estrogen activity; progesterone, uterine weight ratio, water intake rate and uterine vaginal epithelial keratinocytes also showed improvement.

Chemical fingerprints and bioactivities of DBT

Fifty-four chemical peaks were detected in DBT extracts by an HPLC analysis (**Figure 6-7**) and a total of over 100 DBT extracts from various preparations were analyzed (11). Among these 54 peaks, the markers for RA-derived astragaloside IV, calycosin and formononetin, and for RASderived ferulic acid and ligustilide were identified. In analysis of correlation, the identified 54 peak areas together with the contents of total saponins, total flavonoids and total polysaccharides were considered as independent variables. The results of the four bioactivities, namely proliferation and differentiation of MG-63 cells, estrogenic property in MCF-7 cells and anti-platelet aggregation activity, were considered as dependent variables. By analyzing the correlation of these two kinds of variables, coefficients of correlation between the HPLC data of the 57 chemicals and the bioassay data of the DBT extracts were obtained. The values of the coefficients indicate possible relationship of these chemical peaks with bioactivities, where positive values suggest positive effects of chemicals on bioactivities and negative values suggest negative effects. In the assay of MG-63 cell proliferation, astragaloside IV, formononetin, total saponins and total flavonoids are correlated with the bioactivities (**Figure 6-8**). In the assay of MG-63 cell differentiation, formononetin, total saponins and total flavonoids are correlated with the bioactivities. In the analysis of estrogen promoter in MCF-7 cells, ferulic acid are correlated with the bioactivities. Calycosin and total polysaccharides were two very important factors in the assay of anti-platelet aggregation. On the other hand, the amount of ligustilide showed negative effects in all bioassays (**Figure 6-8**). Other

components of DBT, such as those corresponding to peaks 5 to 15, have high correlation coefficients with the bioactivities, but are yet to be identified.

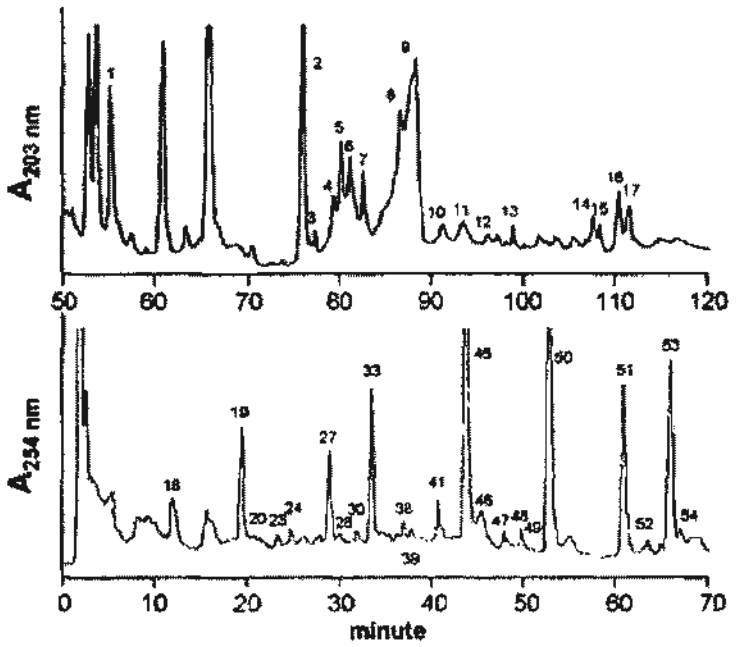


Figure 6-7 Fifty-four peaks in typical HPLC fingerprints of DBT

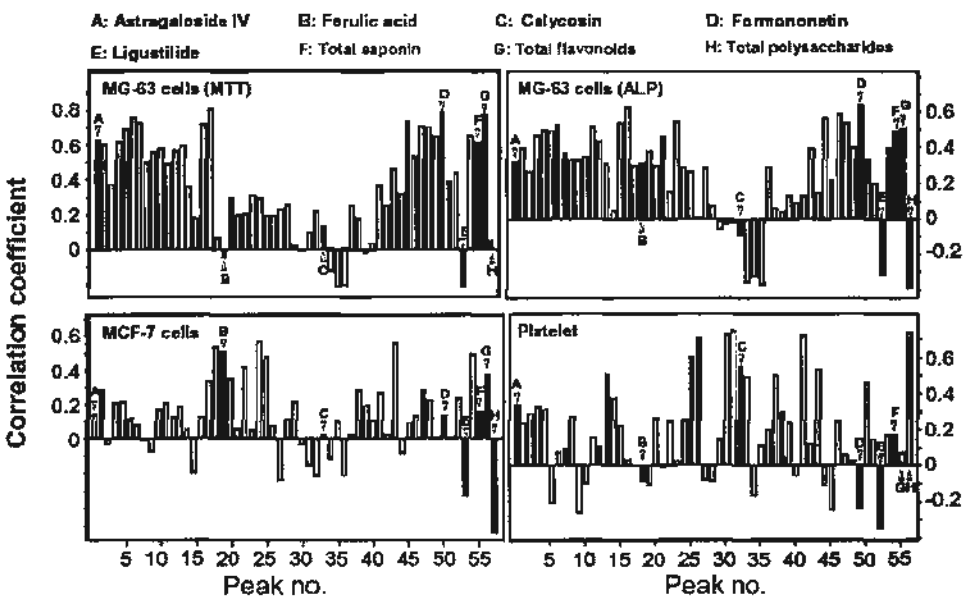


Figure 6-8 Correlation coefficients between the data of 57 chemicals with the four bioassays

Specific estrogenic and immuno-modulatory effects of DBT

The estrogenic effects of DBT were investigated by determining the levels of phosphorylation of estrogen receptor α (ERα) and extracellular signal-regulated kinase 1/2 (ERK1/2) in cultured MCF-7 cells. In contrast to estrogen, DBT triggered the phosphorylation of ERα and ERK1/2 at both S118 and S167 in a time-dependent manner. Although the activity of the estrogen-responsive element in pERE-Luc stably expressing MCF-7 cells was activated by extracts of either RA or RAS alone, or by a mixture of RA and RAS, the phosphorylation of ERα at S167 and of ERK1/2 were only found in DBT-treated cultures.

In cultured T-lymphocytes, the phosphorylation of the ERK 1 (about 42 kDa) and ERK 2 (about 44 kDa) was increased by DBT (22). The induction was transient. An approximately eight-fold increase of ERK phosphorylation was detected 20 minutes after DBT was applied, whereas the phosphorylation was undetectable in the cultures treated with extracts of either RA or RAS alone (15).The phosphorylation of ERK in T-lymphocytes could not be activated by a simple mixture of extracts of RA and RAS. This result suggests that boiling RA and RAS together is essential for DBT to exert estrogenic effects.

Genomics

An experiment on DBT-regulated genes was carried out in our laboratory using gene chip (i.e. microarray) technology. Cultured MG-63 cells were treated with 1 mg/ml of RA, RAS or DBT for 24 hours. The isolated mRNAs were analyzed using microarray, which is a quantitative method to investigate the change of mRNA expression profiles between the control and treatment groups. A total of 8064 genes were screened. Significant changes in gene expression were found after the treatment of DBT, RA or

RAS (Table 3). A total of 883 genes were either up or down regulated by DBT treatment of which 403 genes were DBT-specific; 660 genes were regulated by RA treatment of which 172 genes were RA-specific; 1,062 genes were regulated by RAS treatment of which 473 genes were RAS-specific. In addition, 279 genes were commonly regulated by the extracts of DBT, RA or RAS. The genomic analysis demonstrated not only the activation effect of DBT in stimulating the proliferation and differentiation of the cultured osteoblasts but also a set of candidates of biomarkers that are specifically activated by DBT. These DBT-specific changes in gene expression may be useful in developing biomarkers for quality control of DBT. After identification of these DBTspecific genes and their roles, it will be easier to elucidate the action mechanism of DBT.

6.2 Safety and mechanistic studies of TCM

The risks of a medical intervention for a particular patient, as well as its benefits, should be considered before use. However, benefit–risk assessments for herbal medicines are difficult as information is lacking in several areas relevant to safety.

In the case of herbal medicines, generally, data are lacking on:

- active constituents; metabolites
- pharmacokinetics
- pharmacology
- toxicology
- adverse effects and effects of long-term use
- drug–herb, herb-herb interactions

The insufficient data increase the uncertainty of safety of TCM.

Potential harm can occur via inherent toxicity of herbs, as well as from contamination, adulteration, plant misidentification, and interactions with other herbal products or pharmaceutical drugs.

Pre-clinical toxicology study is of drug safety evaluation; it is a critical part to ensure drug safety. Sensitive parameters of toxic effects proved by long-term toxicity experiment can be used as the scientific basis for clinical toxicity monitoring indicators.

The experience of TCM needs to be substantiated and advanced by the available toolbox of science and technology. The complexity of biological effects of the interactions among different compounds within a decoction complicates experimental studies for revelation of the action mechanisms. Quantitative and qualitative analysis, biological activity, bioavailability, absorption, metabolism, elimination, toxicity, and mode of action studies have been employed using either cell or animal mode.

Widespread favor of TCM also brings serious concern about its safety.

Chinese medicine is derived from animals, plants and minerals. As the differences in soil, water, weather and cultural condition, the toxicity of herbal drugs may have subtle changes) ◦ Under the circumstances of modern industrial civilization, herbal drugs are more vulnerable to be polluted by pesticides, chemical fertilizers and other toxic substances. Some varieties of herbs are confused. Some authentic raw herbs may be mixed with counterfeit goods. These may bring toxic substances. In spite of processing for attenuating toxicity, some toxic herbs are still failed to meet the safety requirements. Some herbal compounds may be incompatible with other drugs; some herbal formulae are added in chemical drugs and some are formed improperly in herbal composition with extremely high dosage. And even medicinal herbs may

acquire toxicity if there is mismanagement in the course of storage and transportation. Some of these errors are due to negligence, some without a clear understanding, however, the main reason is due to very limited information in research in this area.

Toxic Chinese medicine and the content of toxic constituents in Chinese medicine are far from ascertainment. The ingredients of Chinese herbal medicine are extremely complex, which makes the toxicological study more difficult. While in the practical applications of Chinese medicine there are eighteen clashes and nineteen incompatibilities of traditional experiences for reference, but this is not enough. So far there are no comprehensive safety, efficacy and compatibility studies for all herbs related to eighteen clashes and nineteen incompatibilities. Due to lack of knowledge in the toxicity and adverse reactions of traditional Chinese medicine, and without in-depth researches in this area, it is uncertain that some relatively low toxic Chinese herbal medicine used for invigoration and health care maintenance may lead to some safety issues when they are improperly used in the fashion of long-term and large-scale application.

According to records of Chinese Materia Medica, herbs were classified as toxic, non-toxic and the virulence of toxic herbs were further classified as "toxic", "slightly toxic", "extremely toxic" and "deadly toxic", but mostly without objective experimental data. The modern complete concept of toxicity should include acute toxicity, sub-acute and chronic toxicity, specific toxicity (mutagenic, teratogenic, carcinogenic, abortion, addiction) and so forth, but these modern safety evaluation methods have not been fully used in the safety evaluation of traditional Chinese medicine, which influence traditional Chinese medicine in joining the international pharmaceutical market. Although thousands of years' experience has shown that some minerals contained heavy metals, for example cinnabar contained drugs used for treatment of many illnesses are very effective and very safe. However, the basic safety

researches on these kind of drugs were insufficient, and they were not recognized and accepted by international mainstream medicine.

Fortunately, in recent years toxicological studies on traditional Chinese medicine have been carried out to systematically and scientifically evaluate the safety; and Chinese herbal medicines are gradually being recognized and accepted by international market.

Toxicology of both traditional Chinese medicine and western medicine studies the toxic effects and excess drugs on the body, drug induced adverse reactions and toxicity mechanisms, and providing scientific basis for avoiding toxicity and detoxification. Toxicology of Chinese medicine also includes the experimental toxicology, clinical toxicology and toxicokinetics. Toxic reactions of Chinese medicine had brought attention in clinical applications. Toxicity of Chinese medicine was recorded in ancient literatures, especially in recent years toxicity of Chinese medicine has received attention. However, when compared with toxicological study of chemical drug, TCM drugs are far from satisfactory, especially in the compatibility study of "toxic" traditional Chinese medicine. Its unique characteristics and attenuated synergistic mechanism could not be fully understood and has not yet formed a real sense of the toxicology of Traditional Chinese Medicine.

Pre-clinical safety evaluation is intended to provide scientific evidences of new drugs on human body. The purpose of drug toxicology is to study and detect the body damage caused by chemical substances, and its pathogenesis and systemic effects on the body. Pathological examination can determine drug toxicity by means of the pathological lesion site; extent, nature and prognosis, therefore it provides an important basis for the safety of drug. In toxicology studies, most of the tests are conducted in animals. Acute toxicity test, long-term toxicity test, teratogenicity test,

and carcinogenicity test; the toxic location and the mechanism all are relied on the toxicologically pathological examination. The longer the test period, the more important is the pathological result. Therefore, the toxicological pathology is the most important part in pre-clinical safety evaluation.

Any use of herbal products in high dosage or for long-term medication is not advisable unless patients see a practitioner for a diagnosis and holistic approach to their conditions and obtain a customized and personalized prescription, taking the various manifestations of their symptoms and perceived causes into full account. In fact, a carefully prepared combination formula works rather differently, because it contains synergistic and balanced elements that may interact in different ways and neutralize the negative effects of some toxic constituents that the plants might contain. This is the hypothesis, but the public is entitled to have medicines that are proven to work by rigorous tests and that are safe and cost-effective. The standards and the criteria for judging the safety and the effectiveness of treatment and diagnostic interventions must be formulated by critical scientific evaluation from practitioners with different epistemology.

6.2.1 Acute Toxicological Study

The objective of acute toxicity study is to assess the acute toxicity of a test article when administered as a single dose followed by an observation period of 14 days and thereby obtain information both about hazard assessment purposes and for ranking articles.

Single-dose toxicity tests are usually conducted on at least two mammalian species of known strain using equal number of both sexes. Rodents such as the mouse and rat are suitable for the qualitative study of toxic signs and the quantitative determination of

the approximate lethal dose. So the mice are selected as the test model because of its proven suitability in acute toxicity studies.

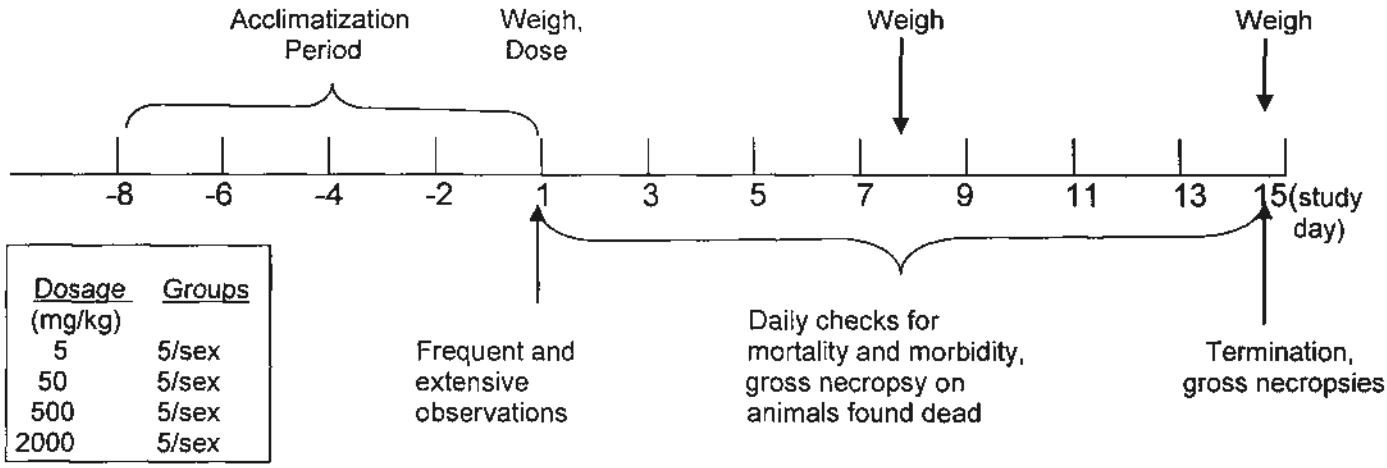


Figure 6-9 Line Chart for the design and conduct of an acute toxicity study

Example of Acute Toxicity Test Report of D&G capsule:

Introduction and Purpose

The purpose of this safety test is to determine whether acute health hazards are associated with ingestion of the test article or not. The measure of acute toxicity can be expressed as the median lethal dose (LD50), a statistically derived value that estimates the dose that would theoretically kill 50% of the test animal group. Such tests require the dosing of a relatively large number of animals to generate precise LD50 values.

Often such a precise measurement of lethality is either not required to characterise the test article or may not be practical as the test article may be minimally toxic to animals following oral administration. To minimise the number of animals used in acute oral toxicity tests without compromising the intent of such safety tests, the use

of limited screening tests with the administration of a single, high limit dose to a group of animals is often adequate for assessing the inherent acute toxicity of the test article.

The test was conducted in accordance with the procedures as outlined in the Guideline issued by Ministry of Health (MOH), Peoples Republic of China (23).

Test Article

Product Description : Capsules for Cardiovascular Tonic

Quantity Received : 0.5g/capsule x 90 capsules/bottle x 30 bottles

SGS Sample No. : 1462043-101

Sample Receiving Condition : In unopened plastic bottle under ambient condition

Sample Receiving Date : 16 December 2003

Test article characterisation (purity, solubility and stability, etc.) was the responsibility of the client. The test article was labelled with laboratory number of this study and kept in room temperature.

Test Animals

Strain : KM mice Clean Animal Grade (male, female)

Source : The Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences

Date(s) Received : 4 January 2004

Upon arrival, an equal number of male and female (n = 20) were randomly assigned to a control group or treatment group. Animals were housed by sex in the observation battery rack and stained the hair on different part of the body for animal identification. Animals were observed for at least 2 days for signs of illness or disease

prior to initiating tests.

Acute Oral Toxicity Test Procedure

White KM mice (male and female, Quality Certificate No.: SCXK-11-00-0006) each weighing between 17 and 19 grams were selected for each dosage. The animals were housed in plastic cages with stainless steel wire mesh caps, the floors of the cages were put with softwood sawdust. The temperature of animal room was 23 2 C, humidity 50 10%. The room was illuminated to give a cycle of 12 hours light and 12 hours darkness. Animals were maintained on a rodent diet provided by The Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences and water was available ad libitum. Twelve hours prior to dosing, all food was removed to fast the animals before initiating the test. On the day of the test, animals were identified and body weights recorded.

The study was initiated with a sighting study, 10 animals (5 females, 5 males) were given the maximum dose 20g/kgbw.

The main study was conducted in 40 animals (20 females, 20 males). In the main study, the test article was administered by oral gavage to groups of 20 female and 20 male mice at 20g/kg.

The dosage to be administered was calculated based on the animal's body weight. The total dosage was up to 20g/kg body weight. The maximum volume is 0.4ml/10g body weight. The control group was treated with same amount of distilled water.

For test articles that are liquids or could be administered as solutions, suspensions or extracts, appropriate doses were administered to animals using a feeding needle and syringe. For certain solid-form test articles, doses were administered by incorporating the material into a feed mix that was fed to laboratory animals over 24 hours period. The method of sample administration used for the submitted test article is outlined in

the Sample Preparation section of this report.

Animals were closely observed for gross toxicological effects immediately after a single dose administration of the sample and then daily for a 7-day observation period. Test animals' body weights, a sensitive indicator of toxic insult, were recorded during the observation period. Necropsies of dead, moribund or surviving animals were performed if indicated during the progression of the study.

Sample Preparation

A test solution was prepared by dissolving the test sample with distilled water to a concentration of 0.25g/ml. A single dose administration of 0.4ml/10g body weight using needle and syringe was given. The control group was treated with same amount of distilled water.

Clinical signs

Each mouse was observed for signs of toxicity at 1, 3 and 6 hours after administration during Day 1 and thereafter daily for a period of 7 consecutive days.

Statistical Analysis

Data were analyzed using student t-test for the body weight with SPSS 10.0 for Window.

Results

40 KM mice (20 male, 20 female) were administered an oral dose of the test article 20g/kg.

Testing Period : 9 – 18 January 2004

In the sighting study, no acute toxicity was found at dose level of 20g/kg, as judged by

clinical signs of toxicity during 7 days and no death was observed.

In the main study, following acute oral administration of the test article at 20g/kg, animals did not show any signs of toxicity or gross behavioural changes. All animals appeared normal throughout the 7-days observation period. They had a normal body weight gain during the study period.

Table 6-7 Change of body weight of animal in the oral acute toxicity study of Capsules for Cardiovascular Tonic

Group	No. of Animals	Dose (g/kg)	Mortality %	Average Body Weight (g)		Body Weight Gain	P Value
				Initial	Final		
Test Group	M 10	20	0	18.3 ± 0.8	29.1 ± 1.0	10.8 ± 0.9	>0.05
	F 10	20	0	18.2 ± 0.8	26.9 ± 1.4	8.7 ± 1.1	>0.05
Control Group	M 10	--	0	18.4 ± 0.7	30.2 ± 1.5	11.8 ± 1.4	
	F 10	--	0	18.3 ± 0.8	27.2 ± 1.5	8.9 ± 1.8	

Observations

After the adminstration of 20g/kg, the animals appeared normal throughout the 7-days observation period. There were no differences of body weights between test group and control group ($P > 0.05$).

Conclusion

When tested as specified, the submitted test article identified by the client as Capsules for Cardiovascular Tonic was considered to be practically non-toxic to laboratory animals following oral administration at 20g/kg (**Table 6-8**).

Table 6-8 Classification of Chemical Toxicity (Oral) From GB 15193.3--94

Category	LD50 (mg/Kg)
Extremely toxic	1.0 or less
Highly toxic	1.0 – 50.0
Moderately toxic	51.0 – 500.0
Slightly toxic	501.0 – 5000.0
Practically non-toxic	5001.0 – 15,000.0
Non-toxic	>15,000

6.2.2 Teratogenicity study

During the last two decades people who apply herbal products have been increasing

globally, and more and more herbal preparations are packaged as function food to be straightforward consumed from food markets in many countries. It is well known that nature herbs as a unique and safe way to treat and prevent pregnant disorders have been clinically used for thousands of years in China. Pregnant women are frequent herbal users, and they may accept herbal therapies to treat disorders during pregnancy (24). Natural herbs and herbal products are used by pregnant women worldwide but there is insufficient evidence to document their safety in embryo development. However, there is little evidence in herbal safety to both fetal and maternal health.

The developmental toxicity testing is designed to provide general information concerning the effects of prenatal exposure on the pregnant test animal and on the developing organism; this may include assessment of maternal effects as well as death, structural abnormalities, or altered growth in the foetus.

We use *Boehmeria nivea (L.) Gaud. (Urticaceae)* as example to elucidate the importance of teratogenicity test for safety evaluation of herbal drug.

Boehmeria nivea (L.) Gaud. (Urticaceae) is a perennial herbaceous plant widely distributed in tropical Asian. It is used as a common herbal medicine for anti-inflammation, anti-bacteria, diuresis, hemostasis, liver protection and antioxidation for many years in China (25, 26, 27). This herb had been found having clinical potential in prevention of miscarriage since ancient times. Recent studies demonstrated that flavone glycosides in roots and rhizomes of B. nivea could inhibit pregnant uterine contraction in mammals, and chlorogenic acid in B. nivea, which has hemostatic activities, might be also linked to the treatment of threatened miscarriage (28). The study was to evaluate the impacts of aqueous extract of B. nivea on embryonic development in mice, and compare with vitamin A, which is a well-known teratogen.

Pregnant mice were randomly assigned into five groups, i.e. mice treated by gavage with distilled water as negative controls (n = 18), with aqueous extract 2 g/kg/day (equal to human daily dose) as low-dose group (n = 18), 8 g/kg/day as middle-dose group (n = 19) or 32 g/kg/day as high-dose group (n = 18) from the Gd 6 to 15, and treated with vitamin A emulsion 200,000 IU/kg on the Gd 7, 9 and 11 and distilled water on the Gd 6, 8, 10, 12-15 as positive controls (n = 18). The feeding volume was based on 0.5 ml for a mouse with 30 g body-weight. The pregnant mice were observed daily for food and water consumption and any sign of toxicity reaction throughout the whole experimental period such as vaginal bleeding, diarrhea, piloerection.

The maternal body-weight was measured on the gestation day (Gd) 0, 6, 12 and 18. The pregnant mice were sacrificed by cervical dislocation on the Gd 18, and their uteri were removed. The maternal uterus with content, liver, kidney and heart were weighed on the Gd 18 after euthanasia. Specimens of the liver, kidney and heart were fixed with 10% formaldehyde and sections were stained with haematoxylin-eosin for histological examination under microscope. The early and late resorptions, live and dead fetuses, and implantations were identified and counted. The fetal body-weight was recorded. All live fetuses were examined for external malformations immediately after sacrifice. Then they were preserved in 95% ethanol for two weeks and prepared for checking of skeletal malformations (29).

There was no maternal death or abortion found in 5 groups throughout the whole experimental period. No obvious disorder was presented in 5-group mice except 2 mice in positive control group with slight tremors after the last administration. Food and water consumption were normal in all groups. The number of pregnant dams and total implantations in all groups were comparable. No significant difference was

found in implantations per dam or percentages of live fetuses among 5 groups. Average fetal body-weight showed no difference among 5 groups.

The rates of resorptions and post-implantation loss in positive control group were 51.38 and 52.17% respectively, and were significantly higher than those in other groups ($P < 0.001$); whereas there was no statistical difference among other 4 groups (4.56-7.14%, 5.39-7.52%) (**Table 6-9**). Complete early resorptions at scheduled sacrifice were detected in two mice in positive control group on the Gd 18.

The rates of external malformed fetuses and skeletal malformed fetuses in positive control group were 34.71 and 47.93%, and were significantly higher than those in other groups ($P < 0.001$); whereas there was no statistical difference among other 4 groups (0.00-0.41% and 4.39-6.91%) (**Table 6-10 and 6-11**). The external malformations in positive control mice were presented as exencephaly and short tail.

The maternal body-weight and gravid uterus-weight in positive control group were significantly lower than those in negative control group on the Gd 18 ($P < 0.05$); whereas there was no statistical difference among other 4 groups (**Table 6-12**). There was no significant difference in relative body-weight gain, liver-, kidney- or heart-weight and relative organ-weight among 5 groups. Meanwhile, no pathological change in liver, kidney and heart samples was observed.

Table 6-9 Fetal parameters in 5-group mice treated with B. nivea extract, distilled water or vitamin A during organogenesis

Parameter	Negative control	Dose (g/kg/day)			Positive control
		2	8	32	
No. of pregnant females	18	18	19	18	18
No. of implantations	241	244	260	266	253
No. of total fetuses	230	229	245	247	123
No. of live fetuses	228	228	241	246	121
% of live fetuses	99.13	99.56	98.37	99.60	98.37
No. of dead fetuses	2	1	4	1	2
% of dead fetuses	0.87	0.44	1.63	0.40	1.63
No. of early resorbed fetuses	9	15	11	15	116
No. of late resorbed fetuses	2	0	4	4	14
No. of resorbed fetuses	11	15	15	19	130
% of resorbed fetuses	4.56	6.15	5.77	7.14	51.38*
No. of post-implantation loss	13	16	19	20	132
% of post-implantation loss	5.39	6.56	7.31	7.52	52.17*
Fetal body weight (g)[a]	1.29 ± 0.10	1.27 ± 0.11	1.31 ± 0.15	1.27 ± 0.10	1.34 ± 0.20

[a] Data were presented as mean ± SD.

* $P < 0.001$ compared with any other group by x^2-test

Table 6-10 Fetal external malformations in 5-group mice treated with B. nivea extract, distilled water or vitamin A during organogenesis

Parameter	Negative control	Dose (g/kg/day)			Positive control
		2	8	32	
No. of fetuses (litters) examined	228 (18)	228 (18)	241 (19)	246 (18)	121 (18)
No. of external malformed fetuses	0	0	1	0	42
% of external malformed fetuses	0	0	0.41	0	34.71*
No. of fetuses with					
Exencephaly	0	0	0	0	26
Short tail	0	0	1	0	16

* $P < 0.001$ compared with any other group by x^2-test.

Table 6-11 Fetal skeletal malformations in 5-group mice treated with B. nivea extract, distilled water or vitamin A during organogenesis

Parameter	Negative control	Dose (g/kg/day)			Positive control[a]
		2	8	32	
No. of fetuses (litters) examined	228 (18)	228 (18)	241 (19)	246 (18)	121 (18)
No. of skeletal malformed fetuses	10	12	14	17	58
% of skeletal malformed fetuses	4.39	5.26	5.81	6.91	47.93*
No. of fetuses with					
Interparietal bone defect	0	0	0	0	26
Parietal bone defect	0	0	0	0	26
Occipital bone defect	0	0	0	0	26
Unossified sternebrae	2	3	3	4	30
Split sternebrae	0	0	0	0	21
Misaligned sternebrae	0	0	0	0	4
Unossified metacarpals	5	4	5	6	8
Unossified metatarsals	3	4	4	5	8
Absence of caudal vertebrae	0	0	1	0	9
Extra ribs	0	1	1	2	4
Absence of ribs	0	0	0	0	6

[a] In positive control group, some fetuses had more than one type of skeletal malformations.
* $P < 0.001$ compared with any other group by χ^2-test

Table 6-12 Maternal parameters in 5-group mice treated with B. nivea extract, distilled water or vitamin A during organogenesis

Parameter	Negative control	Dose (g/kg/day)			Positive control
		2	8	32	
No. of pregnant females	18	18	19	18	18
No. of implantations	241	244	260	266	253
Implantations/dam[a]	13.39 ± 2.09	13.56 ± 2.36	13.68 ± 3.37	14.78 ± 1.99	14.06 ± 2.58
Maternal body weight during pregnancy (g)[a]					
Gd 0	30.30 ± 2.10	30.49 ± 1.91	30.59 ± 2.01	30.66 ± 1.87	30.77 ± 1.99
Gd 6	32.43 ± 2.52	33.51 ± 2.42	33.07 ± 2.39	33.40 ± 2.09	33.75 ± 2.47
Gd 12	36.64 ± 3.33	38.01 ± 3.37	37.52 ± 2.71	38.29 ± 1.42	36.92 ± 3.86
Gd 18	54.77 ± 5.35	55.88 ± 4.11	55.51 ± 5.05	57.02 ± 2.04	48.37 ± 8.63*
Gravid uterus weight (g)[a]	19.75 ± 2.88	19.78 ± 2.62	19.86 ± 3.94	21.04 ± 2.33	12.34 ± 6.94*
Relative body weight gain (g)[a]	4.72 ± 1.82	5.60 ± 2.07	5.06 ± 1.53	5.33 ± 1.99	5.27 ± 2.47
Maternal organ weight (g)[a]					
Liver (absolute)	2.66 ± 0.14	2.69 ± 0.11	2.70 ± 0.21	2.73 ± 0.13	2.67 ± 0.28
(relative)	7.62 ± 0.40	7.49 ± 0.54	7.59 ± 0.58	7.59 ± 0.40	7.43 ± 0.69
Kidneys (absolute)	0.48 ± 0.05	0.48 ± 0.04	0.49 ± 0.05	0.48 ± 0.07	0.49 ± 0.05
(relative)	1.37 ± 0.12	1.33 ± 0.14	1.37 ± 0.11	1.34 ± 0.17	1.37 ± 0.15
Heart (absolute)	0.16 ± 0.02	0.16 ± 0.01	0.16 ± 0.02	0.16 ± 0.02	0.17 ± 0.04
(relative)	0.46 ± 0.05	0.43 ± 0.05	0.45 ± 0.05	0.44 ± 0.06	0.47 ± 0.10

[a] Data were presented as mean ± SD
* $P < 0.05$ compared with negative control group by t-test

Orally administering aqueous extract of B. nivea roots and rhizomes at 16 times (32 g/kg/day) of human daily dose to ICR mice during organogenesis did not cause

embryotoxicity, fetal external or skeletal malformations, and did not induce maternal liver, kidney or heart damage.(30)

6.2.3 Long-term Toxicological Study

Acute toxicity test does not fully capture the safety of Chinese medicine. Because traditional Chinese medicine clinical uses usually cover a longer period, so long-term toxicity test can better reflect the safety of Chinese medicine.

The duration of long-term toxicology studies would depend on the indication of the herbal and the length of the proposed clinical trial. In general, if the herbal medicine is for single administration or repetitive administration spanning less than a week, the test administration period should be 2 weeks to a month; if the medicine is for repetitive administration spanning over a week, the test administration term should be 3~4 times of the period of clinical treatment (usually, not over 6 months in maximum for rodents, and not over 9 months for non-rodents) (31)

In order to understand the safety evaluation procedure of long-term toxicology study, we use DBT as example to elucidate the design and implementation of 90-day toxicity test.

Objective

The objective of this study was to assess the chronic and systemic toxicity of DBT Capsule in the rat when administered for a period of 90 days.

Animals

The experiment was performed in 65 SD rats Clean Animal Grade (32 males and 33 females). At the start of the acclimatisation period, the rats were 5 to 6 weeks old and in the weight range 180-210 g.

The groups, dose levels and animal numbers were as follows:

Table 6-13 Groups, dose levels and animal numbers

Group	Dose levels (g/kg b.wt./day)	Dose volume (ml./100g)	Animal Numbers	
			Males	Females
1	0 (vehicle)	10	1-6	7-13
2	0.6	10	66-79	80-93
3	3.0	10	94-107	108-121

The treatment was given orally by gavage according to the most recent body weight. The animals were treated daily for 90 days and until the day before necropsy.

General observations

All visible signs of ill health and any behavioural changes were recorded daily. Any deviation from normal was recorded with respect to time of onset, duration and intensity.

Body weight

All animals were weighed on arrival, on the first day of treatment (day 1) and weekly thereafter. Also, the weight at necropsy was recorded.

Laboratory investigations

During the last week of treatment, blood samples were taken from all animals. Blood samples were drawn from the tail artery. For haematology approximately 300 µl EDTA stabilised blood was taken. Twenty µl blood was taken with micropipette with dry Na-heparin for the blood glucose analysis. As much blood as possible was taken for clinical chemistry in plain glass tubes for serum. The samples were analysed on the day of collection. Remains of the serum samples were frozen and stored at approx. -18^0C.

Parameters evaluated were the following:

Hematology: blood cell morphology, erythrocyte indices, hematocrit, hemoglobin, mean platelet volume, platelets, red and white blood cells and reticulocytes counts

Table 6-14 Haematology

Parameter	Method/Equipment	Unit
White blood cell count (WBC)	Auto-Hematocyte counter MEK 6318K	10^9/L
Red blood cell count (RBC)	Auto-Hematocyte counter MEK 6318K	10^{12}/L
Haemoglobin (Hb)	Auto-Hematocyte counter MEK 6318K	g/L
Hematocrit (HCT)	Auto-Hematocyte counter MEK 6318K	%
Mean corpuscular volume (MCV)	Auto-Hematocyte counter MEK 6318K	%
Mean cell hemoglobin (MCH)	Auto-Hematocyte counter MEK 6318K	fL
Mean corpuscular hemoglobin concentration (MCHC)	Auto-Hematocyte counter MEK 6318K	pg
LY%	Auto-Hematocyte counter MEK 6318K	g/dL
MO%	Auto-Hematocyte counter MEK 6318K	%
GR%	Auto-Hematocyte counter MEK 6318K	%
Platelet count (Plt)	Auto-Hematocyte counter MEK 6318K	%
Lymphocyte (LY)	Auto-Hematocyte counter MEK 6318K	10^9/L
Mononuclear leucocyte (MO)	Auto-Hematocyte counter MEK 6318K	10^9/L
Granulocyte (GR)	Auto-Hematocyte counter MEK 6318K	10^9/L
RDW	Auto-Hematocyte counter MEK 6318K	10^9/L
PCT	Auto-Hematocyte counter MEK 6318K	%
Mean Platelet volume (MPV)	Auto-Hematocyte counter MEK 6318K	%
PDW	Auto-Hematocyte counter MEK 6318K	fL

Clinical chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, blood urea nitrogen, cholesterol, creatinine, electrolytes, globulin, glucose, total bilirubin and protein

Table 6-15 Clinical chemistry

Parameter	Method/Equipment	Unit
Alanine aminotransferase (ALT)	Automatic Analyzer RA 1000 III	U/L
Aspartate aminotransferase (AST)	Automatic Analyzer RA 1000 III	U/L
Total Protein (TP)	Automatic Analyzer RA 1000 III	g/L
Albumin (ALB)	Automatic Analyzer RA 1000 III	g/L
Total Cholesterol (CHOL)	Automatic Analyzer RA 1000 III	mmol/L
Blood Urea Nitrogen (BUN)	Automatic Analyzer RA 1000 III	mmol/L
Creatinine (Crea)	Automatic Analyzer RA 1000 III	μmol /L
Glucose (GLU)	Automatic Analyzer RA 1000 III	mmol/L
Triglycerides (TG)	Automatic Analyzer RA 1000 III	mmol/L

Histopathological examination:

On the day of necropsy the animals were weighed, examined externally and sacrificed by exsanguination. The animals were sacrificed and necropsied in the sequence of one or two animals/group.

Necropsy: A macroscopic examination was performed after opening the cranial, thoracic and abdominal cavities and by observing the appearance of the organs and tissues in situ. Any macroscopic change was recorded with details of the location, colour, shape and size.

Organs and tissues: Either whole organs or selected samples of the indicated organs and tissues were subjected to the procedures itemized in the list given below. Weights were recorded in computer.

Table 6-16 Organs and tissues

Organs and tissues	Organ / Body weight Ratio	Micro
Abnormalities (gross lesions)	X	
Heart	X	X
Liver	X	X
Spleen	X	X
Lungs	X	X
Kidneys	X	X
Ovaries		X
Testes		X

Paired organs were weighed together. The relative organ weights, i.e. the organ weight as a percentage of the body weight, was calculated for each animal.

All tissues were initially fixed in phosphate buffered neutral 5% formaldehyde with the exception of testes (Bouins fixative). The fixative for long-term preservation was phosphate buffered neutral 5% formaldehyde for all tissues. The lungs were infused with fixative at necropsy.

After fixation, the organs and tissues sampled for microscopic examination were trimmed and representative specimens were taken for histological processing. The specimens were embedded in paraffin and cut at a nominal thickness of approximately 5 µm, stained with haematoxylin and eosin and examined under a light microscope.

Histological alterations were graded on a 5-grade system:

Grade 1 - Minimal/Very few/Very small

Grade 2 - Slight/Few/Small

Grade 3 - Moderate/Moderate number/Moderate size

Grade 4 - Marked/Many/Large

Grade 5 - Massive/Extensive number/Extensive size

Present - Finding present/Severity not scored

Results

Body weights

No significant differences in the mean body weights were observed between treatment groups of either males or females.

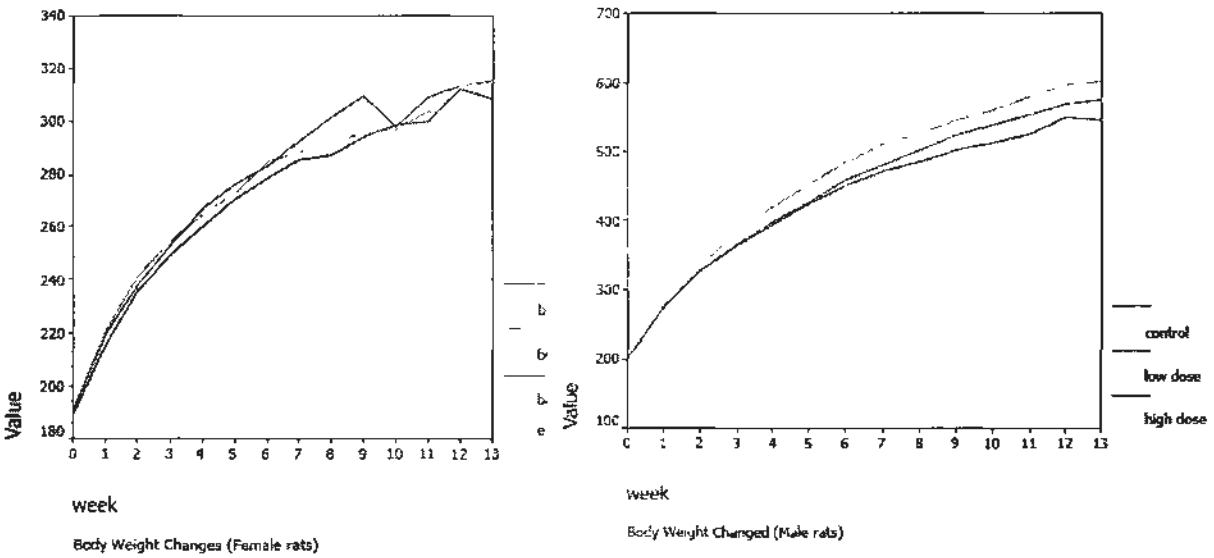


Figure 6-10 Changes in body weight of SD (male and female) rats during treatment with DBT for 90 days

Clinical observations

All animals survived to the end of treatment or recovery, and no general clinical signs treatment related were noted in the treated groups when compared with controls during treatment or the recovery periods.

Laboratory investigations

Haematology (**Table 6-17**) showed that in males, platelets was significantly increased in the 3.0g/kg group, and MCHC value was also increased in comparison with the control group. MCV, HCT, MO% and MO were significantly decreased when compared with the compared with the control group. In females(**Table 6-18**), PCT

was significantly increased in the 3.0g/kg group when compared with the control. Most of those parameters were returned to normal except to HCT, PLT and MO during recovery period.

Table 6-17 Haematological findings of Male rats treated orally with Danggui Capsule for 90 days

Dose (ml/day)	0	0.6	3.0
No. of animals	10	10	10
WBC	18.64±5.41	18.25±3.77	19.54±4.69
RBC	8.66±0.45	8.26±0.51	8.32±0.59
Hb	158.00±7.54	155.50±5.95	157.20±5.29
HCT	51.69±2.99	**47.71±2.43***	**47.57±2.23***
MCV	59.67±1.49	**57.81±2.11***	**57.27±2.51***
MCH	18.25±0.61	18.86±0.85	18.93±1.06
MCHC	306.00±9.07	**326.20±8.53***	**330.60±8.25***
LY%	85.74±8.90	77.21±9.84	77.83±12.87
MO%	4.02±0.64	**2.95±0.59***	**2.89±0.86***
GR%	10.64±8.51	19.84±9.78	19.28±13.38
PLT	253.50±34.99	**297.00±53.02***	**292.60±34.90***
LY	15.87±4.72	14.08±3.51	15.07±3.98
MO	0.77±0.63	**0.55±0.19***	**0.58±0.60***
GR	2.00±1.80	3.62±2.31	3.89±3.50
RDW	12.57±0.62	12.33±0.64	**11.94±0.52***
PCT	0.17±0.02	0.61±0.04*	**0.61±0.03***
MPV	6.95±0.64	7.14±0.19	7.14±0.34
PDW	13.52±0.58	12.97±0.60	13.08±0.54

* $p<0.05$ compared with control group

Table 6-18 Haematological findings of Femal rats treated orally with Danggui Capsule for

90 days

Dose (ml/day)	0	0.6	3.0
No. of animals	10	10	10
WBC	16.66±4.23	16.51±4.25	14.43±3.80
RBC	8.06±0.35	7.85±0.51	7.95±0.40
Hb	155.30±8.19	155.90±6.94	153.90±7.77
HCT	48.47±2.79	46.51±2.66	**46.27±2.02***
MCV	60.15±1.77	59.31±1.05	**58.25±2.00***
MCH	19.28±0.58	**19.89±0.54***	19.37±0.68
MCHC	320.60±6.67	**335.40±7.03***	**332.50±10.75***
LY%	85.32±6.35	84.50±13.62	85.23±9.44
MO%	3.45±0.73	**2.64±0.66***	3.22±1.22
GR%	11.23±6.21	12.86±13.78	11.55±8.44
PLT	247.20±40.69	274.10±43.56	266.30±56.18
LY	14.36±4.50	13.86±4.09	12.17±2.91
MO	0.58±0.19	0.47±0.18	0.49±0.31
GR	1.72±0.93	2.18±2.64	1.77±1.65
RDW	11.75±0.32	11.90±0.34	**12.19±0.39***
PCT	0.17±0.04	0.19±0.03	0.19±0.05
MPV	6.88±0.69	7.13±0.66	7.18±0.32*
PDW	13.39±0.68	13.59±0.56	13.67±0.53

* $p < 0.05$ compared with control group

Clinical chemistry showed (Table 13) that in male, ALB value was significantly decreased in dosing group, and Crea value was significantly increased in low dose group, but no dose-response relationship was noted. During recovery period (**Table 6-20**), ALB in dosing groups and TG in low dose group were still higher than control.

Table 6-19 Clinical Chemistry of Male rats treated orally with Danggui Capsule for 90 days

Dose (ml/day)	0	0.6	3.0
No. of animals	10	10	10
ALT	36.40±7.72	38.50±10.36	39.60±6.90
AST	111.00±22.80	107.60±17.29	114.70±17.08
GLU	6.64±0.95	6.76±0.35	6.75±0.57
TP	63.52±2.87	64.61±1.39	63.83±2.65
ALB	31.22±2.06	**34.08±0.72***	**33.77±1.21***
Crea	76.32±7.15	**87.17±4.82***	81.55±8.80
BUN	5.65±0.85	6.16±0.68	5.59±3.02
CHOL	1.56±0.44	1.72±0.62	1.77±0.08
TG	1.01±0.34	1.31±0.30	1.03±0.68

● $p < 0.05$ compared with control group

Table 6-20 Clinical Chemistry of Male rats Recovery Period

Dose (ml/day)	0	0.6	3.0
No. of animals	3	3	3
ALT	45.33±3.79	47.67±8.08	35.67±1.53
AST	118.67±18.01	101.67±14.84	110.00±22.07
GLU	7.47±0.38	6.92±0.37	6.94±0.67
TP	64.80±2.70	64.87±1.21	65.97±1.59
ALB	30.97±2.10	**34.43±0.68***	**34.83±3.07***
Crea	79.07±6.67	87.47±5.24	85.70±7.86
BUN	5.61±3.01	6.12±3.01	5.35±1.53
CHOL	1.97±0.55	1.83±0.13	1.77±0.06
TG	0.95±0.41	**1.56±0.17***	1.03±0.41

* $p < 0.05$ compared with control group

Gross and microscopic examination revealed no treatment related findings were recorded. No histopathological changes were seen in the livers of 3.0g/kg animals on day 90 of the dosage regimen. No pathological changes were observed in treatment and control groups at the end of treatment period and 14 days recovery period.

No mortality or abnormal signs in behavior, breathing, cutaneous effects, sensory nervous system responses, or gastrointestinal effects were found in the rats treated with DBT at a 3000 mg/kg dose. According to these results, the no observed adverse effect level (NOAEL) of DBT was greater than 3000 mg/kg.

It can be concluded that no treatment related effects were seen in rats after ninety days of oral treatment with DBT at concentrations of 0.6 or 3.0 g/kg body weight/day and fourteen days recovery period.

Chapter 7

Overcoming the Difficulties---Clinical Trials in Traditional Chinese Medicine

A Chinese herbal formula, whether how effective the pre-clinical pharmacology, pharmacodynamics proved, or how safe the animal toxicological studies demonstrated, if there are no clinical trial data to prove its efficacy and safety, the Chinese herbal formula ultimately cannot be considered valid and cannot get marketing approval. Thus, clinical trial plays a decisive role in the research and development of Traditional Chinese Medicine. Currently the highest level of efficacy evidence is obtained from randomized controlled clinical trial. (1, 2). This chapter will take DBT as example to elucidate the clinical trial of traditional Chinese medicine in the protocol design, sample size estimation, statistical considerations, case record form design, subject recruitment, placebo preparation, clinical trial implementation, clinical trial data analysis and clinical trial report writing.

7.1 Importance of Randomized controlled trial (RCT) for Chinese herbal medicine

In clinical research, a well-designed clinical trial protocol is crucial. The methodology for conducting clinical trials should be logical and feasible, and also suitable for Chinese herbal medicines.

Methodology of clinical trials is gradually standardized. TCM Clinical trial mainly focuses on *Zheng*, diseases and both, but basically on disease. Based on "disease" to determine the efficacy is synchronized with the international requirement. The

introduction of the long-term efficacy evaluation undoubtedly enhances the scientific standards of traditional Chinese medicine research. In the selection of key parameters, objective parameters and parameters that mainstream medicine often use should be adopted. Project selection of Clinical trial is basically considered the diseases that are refractory with current medicine. These deficient areas include allergic conditions, autoimmune diseases, cancers, chronic pain, chronic derangements, degenerative diseases, nerve damages, viral infections, and other areas where modern conventional therapy fails. (3)

Clinical trials on Traditional Chinese Medicine usually skip phase I and start with Phase II as the herbs that have been used for a long time of period without safety problem, and the sufficient literatures are available for reference. Using clinical trials to prove the safety and efficacy of such herbal medicines are rarely done, however, it is definitely necessary.

For safety and efficacy evaluation, the evidence from placebo-controlled, double blind randomized trial is considered as gold standard. However, for herbal medicine it may be particularly difficult to do because it needs proper sample size, well-prepared placebo and appropriate parameters.

Efficiency of TCM has relied on 4,000-year's experience

Randomized control trial is regarded as a reliable approach to obtain the strong evidence of efficacy and safety. The classical way to test efficacy of a intervention is clinical trials. It must: compare the effect of treatment with the effect of no treatment; ensure that people are randomly selected into each group; control for placebo effect; identify a clearly diagnosed illness and a standardized treatment; provide for a noticeable change; use measures that are precise, valid and reliable. The most

common version of clinical trials, which satisfy these rules, is the double-blind, randomized controlled clinical trial (RCCT), where neither patient nor the investigator giving the treatment knows who is receiving the actual treatment.

The controlled clinical trial remains the cornerstone for clinical evaluation of traditional medicine for safety and efficacy. However in the field of traditional Chinese medicine there are several constraints in carrying out RCT. But without clinical trials, the efficacy and safety of TCM cannot be demonstrated. The double-blind randomized controlled clinical trial is the 'gold standard' for the measurement of efficacy of clinical intervention.

To scientifically evaluate the efficacy of a Chinese herbal formula, the following issues should be considered:

- Traditional Chinese medicine does not have any history of modern scientific development
- TCM relies on the vast amount of trial-and-error medical knowledge
- Historical value should not be used as the only important evidence of efficacy
- To make use of TCM knowledge in a scientific world, we need to obtain convincing evidence to prove its efficacy
- Current methodology for conducting clinical trial on modern medicine is feasible and acceptable for Chinese medicine
- The proper analysis of data and the statistical methodology should be reliable
- RCT method by means of randomization, blinding, and placebo control followed by proper statistical analysis should be adopted

To gain public trust and to bring herbal product into mainstream of health care system, the researchers, the manufacturers and the regulatory agencies must apply rigorous scientific methodologies and clinical trails to ensure the quality and efficacy consistency of the traditional herbal products. By using modern technologies, the quality and consistency of the heterogeneous herbal products can be monitored. A well-designed clinical trail is necessary to prove the safety and effectiveness of a therapeutic product. The basic principle and design of the clinical trails for herbal products are the same as those for single component chemical product. Thousands of years of traditional use can provide us with valuable guidelines to the selection, preparation and application of herbal formulations. To be accepted as viable alternatives to western medicine, the same rigorous methods of scientific and clinical validations must be applied (4).

7.2 Protocol design of randomized controlled trial (RCT) for Chinese Medicine

The randomized control trial (RCT) is a trial in which subjects are randomly assigned to one of two groups: one (the experimental group) receiving the intervention that is being tested, and the other (the comparison group or control) receiving an alternative (conventional) treatment (**fig 7-1**)(5).

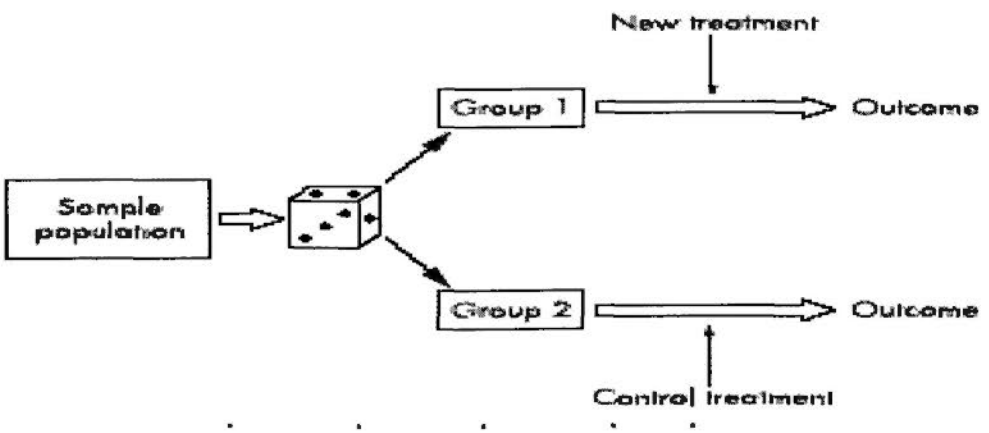
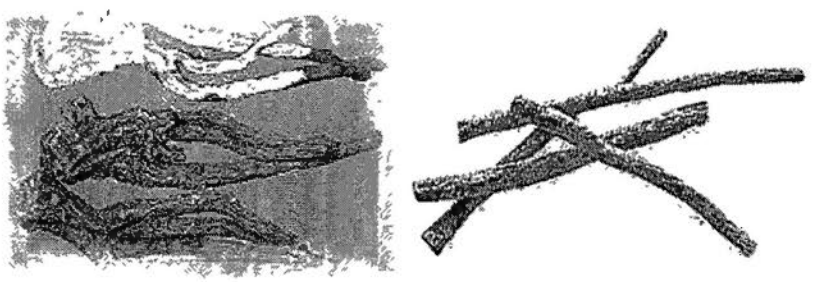


Figure 7-1 The randomized control trial.

The two groups are then followed up to see if there are any differences between them in outcome. The results and subsequent analysis of the trial are used to assess the effectiveness of the intervention, which is the extent to which a treatment, procedure, or service does patients more good than harm. RCTs are the most stringent way of determining whether a cause-effect relation exists between the intervention and the outcome (4).

We take *Danggui Buxue Tang* (DBT 當歸補血湯) clinical trial design as example to elucidate the design of TCM clinical trial.

Danggui Buxue Tang (DBT 當歸補血湯) is a combination of *Danggui* 當歸 (*Radix Angelicae Sinensis*) and *Huangqi* 黃芪 (*Radix Astragali*) in a weight-to-weight ratio of 1: 5 **(Figure 7-2)**. It is one of the simplest classical Traditional Chinese herbal formulas. The formula has been reported in ancient Chinese medicinal literature to relieve blood deficiency in female especially after menstruation or after giving birth.



Danggui 當歸　　Huangqi 黃芪

Figure 7-2 Herbal Formula of DBT

Although Traditional Chinese Medicine (TCM) has a long history but its efficacy is not as well documented as one would hope. Evidence of efficacy should come from well-designed clinical trials. The aim of the study was to prove the efficacy of DBT for menopausal symptoms by establishing and applying rigorous clinical trial methodologies.

This clinical study was consisted of two stages.

The objective of the first trial (**Stage I**) was to examine the effect of *Danggui Buxue Tang* (當歸補血湯) on menopausal symptoms of hot flushes and sweating, and the second trial (**Stage II**) was to investigate the dose response relationship to assess an optimal dose suitable for clinical use.

Stage I trial was designed as a single-center, randomized, double blind, placebo-controlled parallel study. Subjects in **stage I** trial were randomized to one of two treatment groups: Danggui Buxue Tang (當歸補血湯) or placebo (**Figure 7-3**).

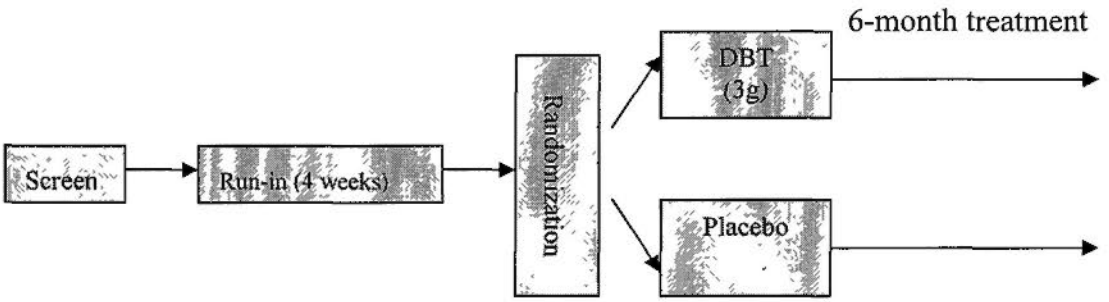


Figure 7-3 Clinical trial schema (Stage I)

In the **Stage II** clinical trial, the objective was to investigate the optimum dose for clinical application. The trial was designed as a multiple-dose escalation clinical trial to obtain accurate information on the efficacy and safety when used for menopausal women. Since stage 1 has already confirmed that the traditional dose (normal) is efficacious, the main purpose of **stage II** trial was to look for an optimal dose for the treatment of menopausal symptoms.

Therefore three groups were used: one group was treated with the normal dose which was same as stage 1 trial. From the normal dose we upgraded 2 times to 6 g daily as high dose and downgraded 2 times to 1.5 g daily as low dose.(**Figure 7-4**)

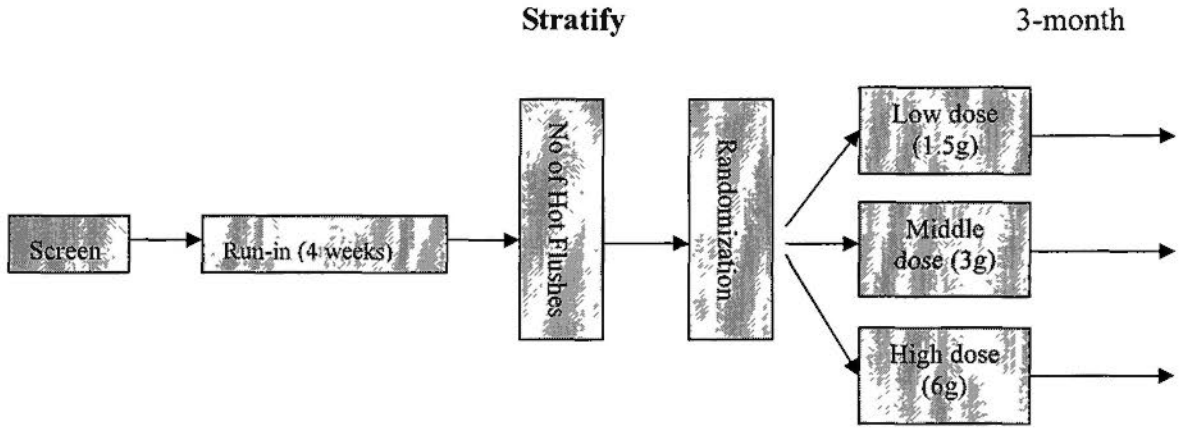


Figure 7-4 Clinical trial schema (Stage II)

Study participants were stratified at randomization into one of two strata (strata 1 = 14-21/week; strata 2 = >21 / week) based on number of hot flushes.

Dose Determination for stage II trial

In TCM, diagnosis and treatment of diseases are based on symptom differentiation principles (辨証論治). The dose of herbal drug is always added or subtracted according to disease status and the dose range is very wide. For example, the textbooks of TCM pharmacy recommended dosage of raw herbs for adults is：lighter dose 3—10g; the heavier dose 10—30g. The variations are very wide (6). In some doctors' prescriptions, the dose range was extremely wide up to several times differences; such as *Wild Jujuba Seed* can be used as high as 100g per dose, but the normal dosage only 10—18g.

Herbal dosage is a concept that is difficult to grasp due to the limitations in our knowledge about the herbs. It is difficult to characterize the relation between drug doses and adverse events or clinical outcomes with adequate precision.

Decoctions were a primary form of therapy described in the ancient text *Shanghan Lun* (傷寒論). For the majority of commonly used herbs, the dose range is 6-15 grams

for one-day dose, with an average of about 10 grams/day.

The purpose of dose-finding study is to determine the optimal TCM dose that is significantly effective. Optimal doses of TCM medication are very important for clinical application. In fact, it is likely that the negative results reveal two things: one is that the initial claims were exaggerated; the other is that the dosages that are commonly consumed involve underdosing. Determining optimal dosages is of utmost importance for achieving efficacy while avoiding significant toxicity.

Previous **Stage I** clinical trial with the dose of 3g (6 capsules) daily has demonstrated the effects of DBT in the treatment of vasomotor symptoms (hot flashes and night sweats). However, the dosage was considered underdosing because it was only relieving mild vasomotor symptoms. The present study would be designed as multi-dose escalation study to find out optimal dose. Three dosage groups were set:

- High dosage: 6 grams daily
- Middle dosage: 3 grams daily, same as previous clinical trial dosage
- Low dosage: 1.5 grams daily

The results of **Stage I** clinical trial indicated that the improvement of vasomotor symptoms usually occurred after 4 or 5 months of DBT treatment. The daily dosage of 3 grams extracted from the mixture of *Dang Gui* and *Huang Qi* in the ratios of 1 to 5 (w/w) is equivalent to 8.8g of raw herbs, based on the extraction rate of 34% provided by Hong Kong Institute of Biotechnology (HKIB). This dosage is only one-fourth of traditional dosage according to the record of 《內外傷辨惑論》, in which the formula was consisted of Dang Gui 6grams and *Huang Qi* 30 grams, total 36 grams for daily dose.

Previous safety studies have demonstrated that both *Dang Gui* and *Huang Qi* have

very low toxicity. The LD50 of *Dang Gui* in mice is 100g /kg via injection, which is 1000 times higher than human dose. Long-term ingestion at the dosage of 6g/kg showed no abnormality in physical activity, food intake, body weight, urine examination, or hematological and pathological examination. This dose is 60 times higher than human dose. Usually, the recommended dosage of *Dang Gui* for human is 5 to 15 grams. *Huang Qi* is also safe, doses as high as 100g/kg of raw herb had been given by gavage to rats with no adverse effects. Oral ingestion of *Huang Qi* decoction (7.5g/kg) cannot be determined in rats. The LD50 in mice for intraperitoneal injection is approximately 40g/kg. The recommended human dose is usually 10 to 15 grams. The maximum dosage of Huang Qi is up to 120 grams. Above evidence indicated that the doses we used in Stage II was safe.

In the Multiple-Dose Escalation Study, the main purpose of the study was to look for an optimal dose for the treatment of menopausal symptoms. Three groups were used: one group was treated with the normal dose which was the same as stage 1 trial; the second group was treated with a dose 100% higher than the normal dose and the third group with a dose 50% lower than the normal dose.

Low dose group: DBT 1.5g daily for 3 months

Middle dose group: DBT 3g daily for 3 months

High dose group: DBT 6g daily for 3 months

Dosage used in Stage I trial was only one-forth of traditional dosage (3g daily), which was demonstrated effective in control of mild hot flushes. Present study has demonstrated that high dose (6g daily) is more effective in control of vasomotor symptoms (**Figure 7-5**) and improvement of quality of life (**Figure 7-6**).

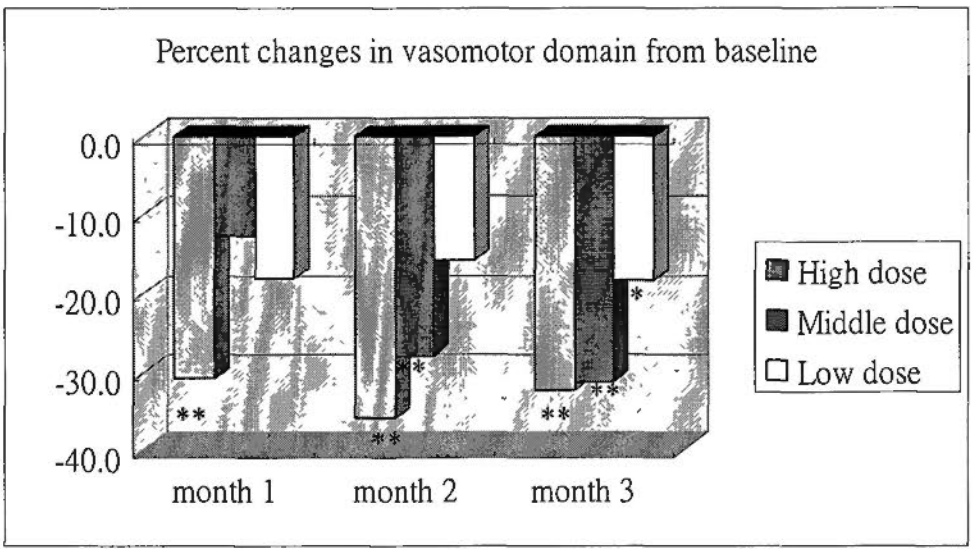


Figure 7-5 Change in vasomotor domain from baseline (**p<0.01, *p<0.05)

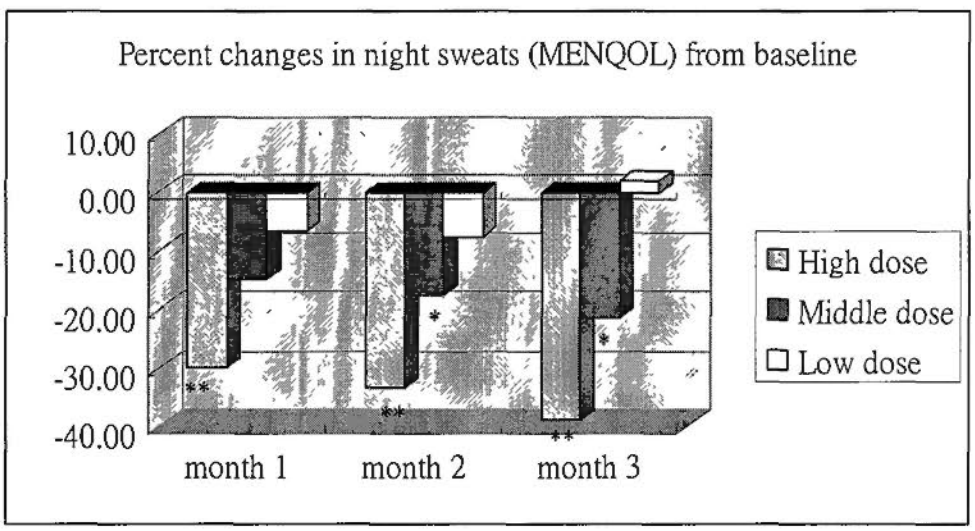


Figure 7-6 Change in night sweat score (MENQOL) from baseline (**p<0.01, *p<0.05)

Dose-effect relationship is one of the important basis in evaluation of adverse reactions. However, the ingredients of Chinese herbal medicine are complex and diverse; they often act on the multi-target and multi-link. Some traditional Chinese medications have no clear dose-effect relationship; furthermore, they have two-way modulatory effects. Therefore, some Chinese herbal medications are not suitable to look for dose-effect relationship. When doing analysis and evaluation of causality, its particularity should be considered.

Rational of the design

Placebo and Double-Blind

Blinding of treatment allocation minimizes the risk of bias during the trial and make sure that the assessment and management of both groups are equivalent. Blinding is important because trial personnel are naturally susceptible to hunches about the effectiveness of one or both trial treatments and only if they are blinded can anyone be confident that decisions and assessments are not affected by such intuitive influences. True double blinding would be optimal, but in practice it remains very difficult, especially for herbal medications.

Randomization and stratification

Randomization took place at the time of the first DBT treatment. A scientifically valid comparison between 2 treatment groups depends on the groups being alike as much as possible, with the only exception being the specific treatments under investigation. The best way to achieve such a balance is by the use of randomization in which a chance mechanism determines the treatment assignment.

The stratified randomization method addresses the need to control and balance the influence of covariates. This method can be used to achieve balance among groups in terms of participants' baseline characteristics (covariates).

For **Stage I** trial, the patients were assigned to receive their allocated treatment according to a computerized generated randomization table prepared by biostatistician:

Group 1: taking DBT 3g daily for 6 months

Group 2: taking placebo 3g daily for 6 months

For **stage II** trial, a computer-generated random numbers table, generated by biostatistician, was also used to assign patients to one of the three treatment arms.

After the patient has been registered into the study, the values of the stratification factors will be recorded, and the patient will be randomly assigned to one of the following treatment groups:

Group 1: Taking DBT 1.5g daily for 3 months

Group 2: Taking DBT 3g daily for 3 months

Group 3: Taking DBT 6g daily for 3 months

For obtaining maximal statistical efficiency, the randomization assigned patients to each group was equivalent i.e. in the ratio of 1:1 (**Stage I**) and 1:1:1 (**Stage II**).

Implementation of double-blind procedure

Quality assessment of a clinical trial methodology not only depends on whether the blind is used, but also how the blind is implemented. Subjects and investigators can not identify the study drug during the course of clinical trial. Placebo in the package, specification, appearance, color, taste, smell and administration method should be fully consistent with the real study drug. Only the placebo that is identical to the study drug can be used in clinical trial.

According to report, during study period some subjects were curious and opened some capsules. They found that they took different drug from other group although they were suffering from same disease. Traditional Chinese medicine can be easily guessed, while the white and tasteless powder was considered to be counterfeit and refused to take, then the trial was failure. In our clinical trial centre clinical trials were usually well designed and prepared, including the placebo preparation. All subjects

were well explained the contents and methods of the clinical trial, so that each of the randomized, double-blind, placebo-controlled trials can be successfully completed.

The randomized controlled trial (RCT) is widely accepted as the most powerful research method in the evaluation of the efficacy of a study medication (7). These principles are also applied to Traditional Chinese Medicine studies. Efficient and effective recruitment of patients and retention of participants are essential steps in the clinical trials. However, it has been shown that the most eligible patients do not participate in clinical trials. There was a study reported that patients who were aware of an appropriate clinical trial, 71% of eligible patients choose not to participate (8). In some RCT clinical trials, the dropout rate was as high as 80% (9). The results of recruitment are generally influenced by a number of factors, including the nature of the study itself, the recruitment strategy used, and the target population itself (10). In this section, we examine some particular barriers to patient participation and explore possible solutions.

Screening and eligibility

After a verbal introduction to the trial, prospective participants were asked several screening questions in interview format, including some eligibility criteria and minimal demographic information. This level of screening was typically done over the phone. All subsequent screening procedures took place in the Center for Clinical Trial on Chinese Medicine, and Department of Obstetrics & Gynaecology, CUHK after providing written consent. Procedures differed depending on whether or not subjects were with menopausal symptoms at the start of screening.

Following an initial vasomotor symptom check, patients with hot flushes 14 times per

week were evaluated for the potential level of hormones and amenorrhoea at least 12 months. For the **stage II** dose finding trial, the potential subjects should be never received treatment for menopausal symptoms and never received menopausal hormone therapy. The evaluation also included a physical exam, clinical history, medication history, Pelvic Ultrasound Endometrial Sampling/ Hysteroscopy, Vaginal Maturation, Arterial Reactivity and assessment of liver function, renal function, lipid profile, urinalysis and a complete blood count.

Additional assessments during screening including self-reported questionnaires to collect information on frequency and severity of hot flushes and night sweats. These questionnaires were completed after consent was obtained.

Eligibility criteria for the randomized trial are listed in **Table 7-1**.

Table 7-1 Eligibility criteria for DBT trials

DBT stage I trial	**DBT stage II trial (dose-finding)**
Inclusion criteria	*Inclusion criteria*
1. Follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol in the menopausal range (FSH>18 IU/L, LH>12.6 IU/L, and E2< 361 pmol/l).	1. Follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol in the menopausal range (FSH>18 IU/L, LH>12.6 IU/L, and E2< 361 pmol/l)
2. Patients with amenorrhoea for more than 12 months	2. Patients with amenorrhoea for more than 12 months
3. Patient with at least 14 hot flushes or night sweats per week	3. Never received treatment for menopausal symptoms
4. Patient able to give written or witnessed oral informed consent prior to study star and able to comply with study requirements	4. Never received menopausal hormone therapy
Exclusion criteria	5. Reporting a minimum of 14 hot flushes per week at the time of entry into the study
1. Patients with a history of using any form of hormonal replacement therapy within 8 weeks	6. Patient able to give written or witnessed oral informed consent prior to study star and able to comply with study requirements
2. Patients with a history of using Chinese medicine or other therapies which may affect the outcome within 8 weeks	*Exclusion criteria*
3. Patients who in the judgment of the investigator will be unable to comply with protocol requirements	1. Patients with a history of using Chinese medicine or other therapies which may affect the outcome within 8 weeks
4. Patients with significant gastrointestinal, renal, hepatic, bronchopulmonary, neurological, cardiovascular, breast or endometrial carcinoma, or allergic diseases	2. Patients who in the judgment of the investigator will be unable to comply with protocol requirements.
5. Patients with uncontrolled hypertension	3. Patients with significant gastrointestinal, renal, hepatic, bronchopulmonary, neurological, cardiovascular, breast or endometrial carcinoma, or allergic diseases
6. Patients with undiagnosed vaginal bleeding	4. Patients with uncontrolled hypertension
7. Patients with a history of significant drug hypersensitivity	5. Patients with undiagnosed vaginal bleeding

Experimental intervention

Among thousands of traditional Chinese herbal formulae, almost all of which consist of multiple herbs, DBT is one of the simplest. Containing only two herbs, namely

Radix Astragali (RA) and *Radix Angelicae Sinensis* (RAS), DBT is traditionally used to treat ailments in women.

According to TCM theories, DBT replenishes *qi* and nourishes *xue* (the blood). DBT is therefore used for treating menopausal symptoms (11).

Control intervention

The protocol design for TCM clinical trial was based on principles of Randomized Controlled Trial (RCT), placebo control was involved, which was designed to be inactive. The purposes of using placebo were to control the bias caused by psychological or non-specific effects of treatment and reduce the chance of being recognized by subjects and investigators during the period of clinical trial. To maintain blinding, the placebo was designed to appear smell, and taste indistinguishably from the trial preparation while pharmaceutical activity and toxicity were both absent. However, if a placebo is easily identified, whether a double-blinded design could be implemented is doubtful, because subjects dislike being assigned the placebo group and the quality of the clinical trial will be affected (12, 13).

The efficacy and dose finding studies were total 9-month long. Efficacy study was 6-month long and dose finding study 3-month long. The "blinded" doctors saw the patients on months 0, 3 and 6 in stage I efficacy trial (**Table 7-2**); and on months 0, 1 and 3 in stage II dose finding trial. The last month was follow-up period without any medication to see whether a rebound would occur (**Table 7-3**).

The medications administered were herbal (DBT) and placebo (stage I) in capsule and three dose levels of DBT in sachets (stage II). All herbs were screened for arsenic and heavy metal contamination. No products were from animal origin or endangered species. Patients in stage I trial were instructed to take 3 capsules and two times a day.

Patients in stage II were instructed to take one sachet a day.

Table 7-2 Schedule of treatment and study assessments (**Stage I trial**)

Period	**Screening**	**Treatment**		
Visit	**0**	**Baseline**	**2**	**3**
Day	-30 to -1	0	91	182
Medical History	X	X	X	X
Menopausal Symptoms	X	X	X	X
Vital Signs	X	X	X	X
Physical Examination	X			
Review of Incl./Excl. Criteria Study	X			
Informed Consent[a]	X			
Urinalysis	X		X	X
Concomitant Medications	X	X	X	X
Randomization		X		
Hormone assay	X		X	X
Hematology	X		X	X
Biochemistry	X		X	X
Pelvic Ultrasound Endometrial Sampling/ Hysteroscopy	X			
Vaginal Maturation	X			X
Body Mass Index (kg/m^2)		X	X	X
Cardiovascular markers		X	X	X
MENQOL[c]		X	X	X
Arterial Reactivity		X	X	X
Dispense Study Medication		X	X	
Medication Accountability			X	X
Record Adverse Event			X	X

Table 7-3 Schedule of treatment and study assessments (**Stage II trial**)

Period	Screening/ Run-in	Treatment			Follow-up
Visit	**0**	**Baseline**	**2**	**3**	**4**
Day	**-30 to -1**	**0**	**31**	**92**	**120**
Medical History	X				
Menopausal Symptoms	X	X	X	X	X
Vital Signs	X	X	X	X	X
Physical Examination	X				
Review of Incl./Excl. Criteria Study	X				
Informed Consent[a]	X				
Urinalysis	X		X	X	X
Concomitant Medications	X	X	X	X	X
Randomization		X			
Hormone assay	X		X	X	X
Hematology	X		X	X	X
Biochemistry	X		X	X	X
Pelvic Ultrasound Endometrial Sampling/ Hysteroscopy	X				
Vaginal Maturation	X			X	X
Body Mass Index (kg/m^2)		X	X	X	X
Greene Scale	X	X	X	X	X
MENQOL[c]	X	X	X	X	X
Dispense Study Medication		X	X		
Medication Accountability			X	X	
Record Adverse Event			X	X	

7.3 Outcome measurement

Outcome selection

In clinical trials, an outcome is defined as a variable intended for comparison between groups in order to assess the efficacy or harm of an intervention (14). Outcomes are

also commonly referred to as endpoints or variables.

TCM treatment usually underlines the holistic view, according to which the manoeuvre for a disease focuses on modulating and improving the entire function of the human body. TCM medication therapies usually consist of a variety of component, with multi-target and multilevel actions. Therefore, therapeutic responses usually reveal a "multi-effect". Under such circumstances, the criteria for outcome measures must be multidimensional in order to assess the efficacy of treatment.

In recent decades, people's understanding of health has a fundamental change. World Health Organization (WHO) proposed the contents of health which include mental health, Social life, and physical health. Improving quality of life of those patients suffering from incurable chronic diseases has become the main purpose of clinical treatment. Traditional Chinese medicine emphasizes the concept of "holistic approach". Chinese medicine does not directly target against a symptom or pathology, but emphasizes the maintenance of harmony between the vital forces of an individual. Quality of life assessment can be used to evaluate patients' rehabilitation by placing them in social and natural environment, which is better in reflecting the characteristics of traditional Chinese medicine. (15)

According to the definition of Quality of Life (QoL), it is a multidimensional index, which reflects overall health status. It is of great importance that the concept and measurement of QoL is introduced into TCM clinical research and practice. When using TCM therapeutic regimens, we may find that taking the QoL as an endpoint criterion is applicable to providing evidence that may reveal the possible improvement of QoL by TCM in a spectrum of diseases.

Outcome measures can be broadly divided into four categories: physician-based, patient-reported, economic-based, and technology-based outcomes (16). Commonly used outcomes in RCTs of TCM can be categorized into TCM specific outcomes such as tongue and pulse characteristics, and Western medicine (WM) specific outcomes such as blood tests and radiographic examination results. Some studies specially conducted in mainland China include both types of outcome measures.

Using the wrong outcome measure can distort the results of a clinical trial and have serious repercussions when making recommendations for clinical practice. If the outcome measure fails to capture the intended changes in health or is not sensitive enough to determine clinically meaningful differences, important information about the efficacy of an intervention would be missed (17).

Endpoints are set to give objective standards for evaluating the effectiveness

Selecting the appropriate outcome measure is very important for an RCT because this is the yardstick against which success is measured and by which conclusions are reached with regard to the efficacy of the intervention under scrutiny. Therefore, careful, thorough consideration in choosing appropriate outcome measures is a prerequisite for obtaining valid, clinically useful evidence from RCTs.

With regard to the selection of outcome measures, the Draft Consolidated Standards for Reporting Trials of Traditional Chinese Medicine (CONSORT for TCM), published in Chinese and English in 2007 (18, 19), suggested that one or two endpoint outcomes (e.g. mortality and survival) for which the definitions are the same in both TCM and conventional medicine should be selected as the primary outcomes.

For example in DBT clinical trial, we selected menopausal symptoms of hot flushes and sweating as primary objective by measuring the changes in severity and

frequency of hot flushes and sweats as primary endpoint. This design was in line with international standard in the field and the results would be convincing.

Because the indication of one herbal formula is a specific pattern, not a disease, stratifying a disease into several patterns is a feasible procedure in clinical trials. Endpoints measured should include specific modern parameters, other associated indices and general improvement (i.e. quality of life or accumulated scores of symptoms) [20]. Despite its difficulties, conforming to both traditional diagnostic and therapeutic systems and modern methodological demands is achievable [24]. TCM is typically used for difficult, but normally non-life-threatening, conditions such as back pain, allergies, arthritis, insomnia, sprains, strains, headache, depression and chronic fatigue. Quantitative standardization of pattern diagnosis is also a good method to re-evaluate the efficacy of herbal formulae.

The importance of primary outcomes in study design

The primary outcome variable is the single (or few) outcome(s) that form the basis for study design. The primary outcome must be predetermined by the investigator when designing a study. This is important because the type of primary outcome measure employed will determine the number of subjects required and the planned statistical analysis for a study. In to estimate sufficient subjects enrolled in a study and detect a difference between study groups, investigator should perform a power calculation. The primary outcome is the fundament for power and sample size calculations.

Problem with multiple primary outcome variables

Multiple primary outcomes are quite common in TCM clinical trials because of the nature of TCM therapy, i.e., holistic approach. The problem with having more than

one primary outcome variable is that with increasing numbers of primary outcome variables, there is a higher chance of achieving a difference between study groups by chance alone, rather than because of a true therapeutic benefit.

Primary End Points

Selection of the primary end point is a key design element of a RCT. Primary end point is the outcome measure used to make the decision on the overall result of the study and serves as the basis to determine the number of patients needed for the study. The primary treatment comparison was to test the efficacy of TCM (for example, DBT versus placebo) whether DBT has an advantage over placebo control on menopausal symptoms of hot flushes and sweating.

Other treatment comparison was to evaluate the safety of *Danggui Buxue Tang* (當歸補血湯) in patients with menopausal symptoms.

So that in DBT clinical trial the treatment comparison was made for each of the several endpoints:

- Changes in severity and frequency of hot flushes and sweats from baseline to 6 months (primary endpoint)
- Changes in score for the domains measured in the Menopause Specific Quality of Life Questionnaire
- Changes in self-reported menopausal symptoms

Primary endpoint is the changes of average number of hot flushes per 24 hours measured over a 4-week period. The numbers of hot flushes were recorded in a daily diary, and flushes were scored by severity on a scale from zero to ten.

Secondary endpoint is the Women's Health Questionnaire (MENQOL), a validated, self-administered instrument containing 36 questions assessing a wide range of

physical and emotional symptoms of women in the postmenopausal period.

Secondary outcome variables

Secondary outcome variables are frequently derived from questions of interest to the investigator, and may form the basis for further study. Secondary outcomes do not play a role in considerations of sample size (power). Consequently, there is no limit to the number of secondary outcomes.

For example in our completed osteoporosis clinical trial, Lumbar spine (L1-L4) BMD determined by dual-energy x-ray absorptiometry was served as primary outcome, the secondary outcomes were included:

Biochemical markers of bone turnover:

- Bone formation: bone specific alkaline phosphatase (ALP),
- Bone resorption: serum N-terminal telopeptide

Drug safety:

- Hematology: erythrocyte, leukocytes, differential count, hematocrit, hemoglobin, and platelets
- Biochemistry: glucose, urea, aspartate transaminase, alanine transaminase, alkaline phosphatase, total bilirubin, creatinine, total protein, albumin, sodium, potassium, chloride, calcium and phosphorus
- Urinary analysis: glucose, protein, blood
- Physical examination: A complete physical examination of each participant will be performed at screening visit, 6-month visit, and 12-month visit.
- Adverse events: At baseline, 3-month (week 12), 6-month (week 24), 9-month (week 36), and 12-month (week 48), adverse events will be recorded. Definition of adverse event please refers to Section 9.1 of the protocol.

Quality of life (QoL)

Considerations in choosing outcome variables

Selecting the most appropriate outcomes in an RCT of TCM remains controversial (21). Most of the concerns about assessing the efficacy and effectiveness of TCM have focused on the selection of TCM specific indicators to reflect the syndrome or pattern. TCM specific indicators may be not accepted by modern medicine. There are still controversial. We believe that objective indicators may be more accepted by mainstream medicine.

Measurability of parameter

The parameters selected for clinical trial should be measurable. This is precisely the weakness of traditional Chinese medicine clinical research because the efficacy parameters of traditional Chinese medicine usually derive from the patients' subjective feelings and doctors' subjective judgments, and transform them into a non-measurable grade, such as improvement, recovery, deterioration etc. In our previous TCM clinical studies we often used measurable parameters. For example, in a clinical study of using Chinese herbal medicine as adjuvant therapy for prevention of osteoporosis, we selected bone mineral density (BMD) as the main OUTCOME MEASURES. In the clinical study of traditional Chinese herbal formula for treatment of diabetic foot ulcers, we selected the wound area and wound healing time as the primary endpoints. In the clinical study of *Danshen* and *Gegen* (D&G) capsule on the cardiovascular effects, we used carotid intima-media thickness (IMT) as the main OUTCOME MEASURES. Another example is the clinical research of DBT for post menopausal syndrome, we used the frequency of flushes and night sweats by times per week as MAIN OUTCOME MEASURES. All these parameters were measurable

and accepted by mainstream medicine.

Objective response

Objectivity of parameters is critical. This is also the weakness of traditional Chinese medicine clinical research. Laboratory tests are objective such as BMD, IMT, wound area, hot flushes etc. These should be clearly defined, and stated in a manner that will allow the objectives to be investigated by a quantitative assessment of appropriate outcomes.

Overall response

Systemic parameters such as quality of life (QoL) for example SF-36 can reflect the overall health status especially for clinical trial of traditional Chinese medicine because it is "holistic approach".

Duration of response

In time-bound parameters such as wound healing of diabetic foot ulcers, the duration of healing is used as primary endpoint to determine the efficacy.

Surrogate outcome measures

A surrogate outcome measure is a laboratory measurement or a physical sign used as a substitute for a clinically meaningful outcome that measures directly how a patient feels, functions, or survives (22).

Surrogate outcomes can be misleading. For example, a study on the treatment of osteoporosis with fluoride supplementation resulted in an increase in bone density (the surrogate marker) and, thus, a "good" outcome. The primary clinical outcome, however, of reduction in fractures did not occur; in fact, they increased (23). How do

we know whether a surrogate outcome is reliable and valid to predict the primary outcome of interest?

The ideal setting for a valid surrogate marker is when the surrogate is in the only causal pathway of the disease process, and the intervention's entire effect on the true clinical outcome is mediated through its effect on the surrogate (**Figure 8-7**).(16)

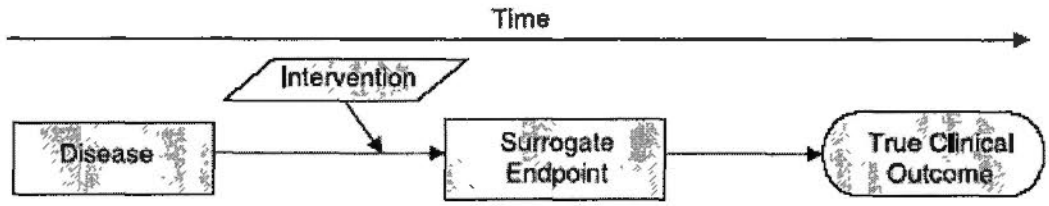


Figure 7-7 Surrogate endpoint and clinical outcome

Clinical relevant end point and efficacy parameters should be sensitive, specific, reliable, and internationally recognized rather than only rely on intermediate indicators (surrogate endpoint) unless there is direct evidence that the intermediate indicators and surrogate endpoint exists a direct correlation.

When conducting a clinical trial of TCM preparation *Rehassure* for the treatment of hepatitis B, we found that in clinical trial of chronic viral hepatitis the evaluation criteria of efficacy are extremely non-uniform. There was "*Clinical Research Guidelines for Traditional Chinese Medicine in Treatment of Viral Hepatitis* "issued by the Ministry of Health, in which the evaluation criteria of chronic hepatitis were divided into: basic cure, effective and ineffective. The later revised version divided into currently cured, markedly effective, improved and invalid, changed to markedly effective, effective and ineffective. There was also "*Viral Hepatitis prevention and treatment programs*" (1991) developed by the Conference, National Professional Institutes of Academic Infectious and Parasitic Diseases (2000), which is divided into

clinical recovery, effective and ineffective. There was another guideline "*Efficacy Criteria of Viral Hepatitis Treated with Traditional Chinese Medicine*" (1991) developed by Traditional Chinese Medicine Society of Internal Medicine Professional Committee of Liver Disease, which was divided into basic clinical cure, markedly effective, improved and ineffective.

All of these grades have adopted comprehensive evaluations, including symptoms, signs, liver function, viral replication, and integrated into above efficacy grading. However, these grades cannot be in line with international standards because of their poor accuracy and objectivity.

Selection of efficacy parameters in Western medicine for chronic hepatitis clinical trial is based on its specific action mode of the study drug, such as the antiviral lamivudine which mainly acts on viral replication; immunomodulator thymosin mainly influences the parameters of immune function, such as lymphocytes. Even with a comprehensive index, it should be separately reported the efficacy of the treatment, such as the efficacy endpoint of the anti-viral (viral replication standard), Liver (ALT), signs and symptoms (scale), reduced liver fibrosis (liver biopsy histopathological changes), etc.

7.4 Statistical considerations

Development of statistical analysis plan

Statistical analysis plan (SAP) includes three parts, which are sample size estimation, statistical methods, and population for analysis.

In sample size estimation, description of the statistical methods for determining the sample size for the study should be given. In statistical methods, summarization of the

overall statistical approach to the analysis should be described. The population for analysis should be very specific to define. Examples of such populations include:

- All-randomized population (Intent-to-treat analysis): Any subject randomized into the study, regardless of whether they received study drug. Subjects may be excluded who never received treatment and/or without follow-up data.
- Protocol-compliant population (Per protocol analysis): Any subject who was randomized and completed major amount of the protocol and follow-up measurements available. No major protocol violation.

We use Benign Prostatic Hyperplasia (BPH) clinical trial as example to elucidate SAP.

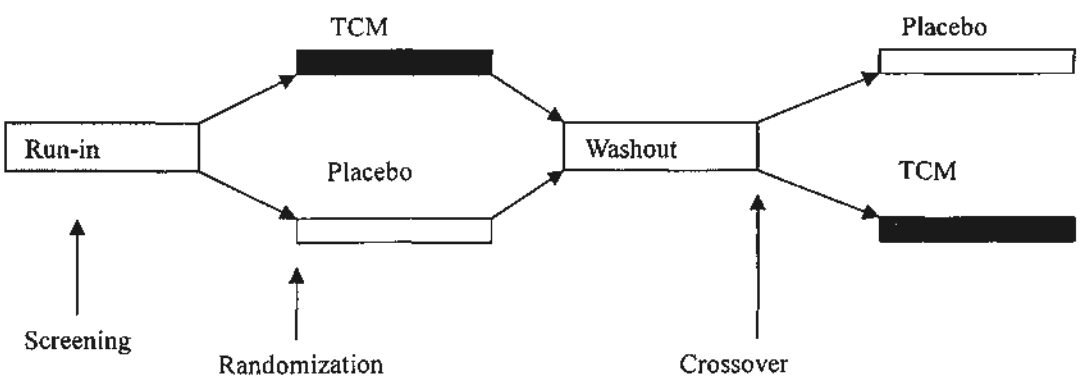
Statistical analysis plan

Title: The Effectiveness of Saw Palmetto and Sanmiaoshan on Benign Prostatic Hyperplasia in Chinese Patients

Study Objective

The primary objective of the study is to investigate the combined effects and safety of Saw palmetto and Sanmiaoshan on BPH symptoms.

Design of the clinical trial



Run-in	Period A	Crossover	Period B
Screening	24 weeks treatment	8 weeks washout	24 weeks treatment

Figure 7-8 Design of the clinical trial

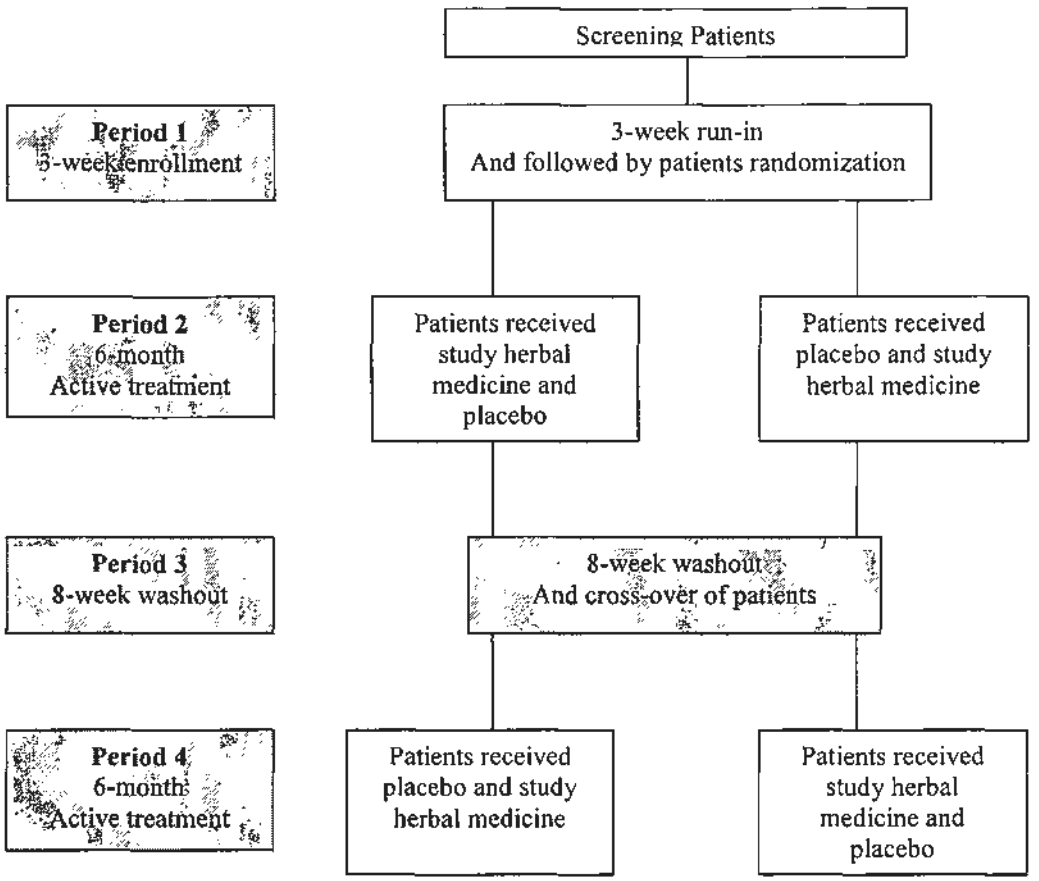


Figure 7-9 Flowchart of the clinical trial

Table 7-4 Treatment schedule

Visit	1	2	3	4	5	6
Week	0	12	24	32	12	24
Treatment Regimen	Saw palmetto + Sanmiaoshan			Washout Period	Placebo	
	Placebo				Saw palmetto + Sanmiaoshan	

Group 1 patients received the treatment sequence of TCM first, crossing over to placebo, and group 2 patients received the reverse treatment sequence, placebo followed by TCM.

Study Endpoints

Changes in BPH symptoms.

1) International Prostate Symptom Score (IPSS)

- Total IPSS
- Obstructive IPSS
- Irritative IPSS

2) QOL (GHQ-30, 30-item General Health Questionnaire)
3) Prostate volume
4) Urinary flow rate (Q-max)
5) Post-void residual volume
6) Laboratory test
    - PSA (prostate specific antigen)
7) Adverse events

Statistical methods used for data analysis

Baseline measurements are required for inclusion in the analysis of change from baseline data. Baseline comparisons are made using a 2-way analysis of variance with effects for treatment group assignment and country for continuous variables. For post baseline assessments, the analysis is carried out at each post treatment visit and at the final visit.

Efficacy data at the end of the first 24 weeks of treatment are evaluated using a *t*-test. Efficacy data of treatment for both treatment periods are evaluated using an analysis of covariance (ANCOVA) model. The model includes baseline as a covariate, with treatment, phase, and sequence within patient as sources of variation. For within-treatment changes from baseline, a paired *t* test is performed. A p value of <0.05 is considered statistically significant.

Descriptive statistics on age, duration of BPH, IPSS, Qmax, mean urinary flow rate, post-void residual volume, prostate volume, PSA and GHQ score will be computed to check consistency and normality of the data. Descriptive statistics on these variables without outlier will then be performed to test the effect of outlying data on overall results. Outliers will be excluded from the analysis if they have great effect on overall statistics.

The power of washout period is analyzed by comparing values recorded in run-in and washout period in patients who finished crossover by paired student's t-test. Significant difference, if any, between run-in and washout period would indicate that the washout period was not adequate.

Data were analyzed using Student's t-test for between-group comparisons of continuous variables, while Chi-square test or Mann-Whitney U test was used for comparison of categorical variables. Paired t-test or Wilcoxon signed rank test was used for within-group comparisons. For all tests, a p value of < 0.05 was considered significant.

1 Baseline characteristics

Baseline values are compared by student's t-test or chi-squared test to ensure no significant difference in these variables between 2 treatment groups.

2 Efficacy Evaluation (IPSS, IPSS QOL)

IPSS, Qmax, mean urinary flow rate, post-void residual volume, prostate volume, PSA and QOL score at baseline among subjects included in and excluded from the analysis will be compared by using t-test for normality data and Mann-Whitney-U test for skewed data. Reliability of the Chinese version of IPSS and QOL will be assessed by using Cronbach's alpha.

IPSS, Qmax, mean urinary flow rate, post-void residual volume, prostate volume and PSA at baseline and after the washout period will be compared to test homogeneity. Adjustment will be made for test on efficacy of the treatment if significant difference is detected.

The outcomes are the differences in IPSS and Qmax between the study medication and placebo groups. The differences in IPSS and Qmax between (1) study medication group and baseline and (2) placebo group and baseline are the secondary end points. The mean urinary flow rate, post-void residual volume, prostate volume, PSA and QOL score between (1) study medication group and placebo group, (2) study medication group and baseline, and (3) placebo group and baseline are also considered as secondary end points

Differences in IPSS, Qmax, mean urinary flow rate, post-void residual volume, prostate volume, PSA and QOL score will be assessed by paired t-test for normality data (Wilcoxon's sign rank test for skewed data).

3 Blood chemical data analysis

Student's t-test and ANOVA were used to compare the differences in results.

4 Adverse events

χ^2 test was used to compare the incidence between the treatment groups.

All analyses were conducted blinded to group allocation. All analyses were done on an "intention-to-treat" basis; i.e. all participants who reported receiving study drugs and taking them at least once would be included. The primary analysis was comparing mean changes from baseline to end of treatment in the two groups, using a two-sample t-test and paired t-test. Comparisons to placebo were conducted using an ANCOVA with baseline as a covariate.

The mean daily number and severity of hot flashes were compared among treatment groups for each month using an ANCOVA, with treatment as a factor in the model and baseline as the covariate.

Secondary analyses compared the changes of other parameters at different time points.

Subgroup analyses include comparison of changes in hot flush rate among women stratified by age and menopausal duration.

Differences in categorical data between groups were explored by Mann-Whitney Test, Wilcoxon Signed Ranks Test or χ^2 test.

Statistical power and sample size

Sample size is primarily important due to its effect on statistical power. Understanding the direct relationship between sample size and power is critical in interpretation of the conclusions that were drawn from a study (25).

A clinical trial report containing sample size estimation or not is important for quality evaluation of the report. In the protocol design phase, the sample size should be determined. In theory, the efficacy validation of an intervention between treatment group and control, the larger sample size the closer to the true, and the results more

reliable. However, because of resource constraints and ethical consideration, the number of subjects in clinical trials can not be infinitely large, it is necessary based on the statistical principles to determine the optimal sample size.

If the sample size is too small, it's a waste of time doing the study as no conclusive results are likely to be obtained. On the other hand, if the sample size is too large, extra subjects may be given a therapy which perhaps could be proven to be non-efficacious with a smaller sample size (26). To estimate a sample size which will ethically answer the research question of an RCT with a reliable conclusion, the following information should be available: minimum expected difference (also known as the effect size), estimated measurement variability, desired statistical power, significance criterion, and one- or two-tailed statistical analysis planned.

Table 7-5 Sample size requirement in each arm to achieve described power for defecting differences specified (binary end point, α=0.05) (27)

Event rate of comparator arm	% reduction in the Test arm	Power	
		0.90	0.80
5%	50%	1291	984
	40%	2115	1605
	30%	3931	2970
10%	50%	621	474
	40%	1014	771
	30%	1881	1422
15%	50%	398	304
	40%	648	493
	30%	1197	906
20%	50%	286	219
	40%	464	354
	30%	855	647

Table 7-5 shown that more power with higher event rate, the less sample size was required. Otherwise, sample size requirements were higher.

The following is the example of sample size estimation for 適鼻靈(SBL) clinical trial.

Title of Study:

A Herbal Formula 適鼻靈(SBL) for the Treatment of Perennial Allergic Rhinitis: A Randomized, Double-blind, Placebo-controlled Clinical Trial

Objective:

Primary:

1. To evaluate the symptoms score, including rhinorrhea, nasal obstruction, sneezing, itchy nose, and itchy eyes before and after treatment.
2. To evaluate the changes in peripheral blood of total Ig E and Der p specific Ig E, eosinophils, basophils, ECP and some related cytokines.

Secondary:

1 To evaluate the quality of life (QOL) questionnaire before and after treatment.

2 To evaluate the safety of the formula to patients by routine blood cell count, biochemistry test and urinalysis test.

Design:

A single-center, randomized, double blind, placebo-controlled study. Subjects will be randomized to one of the two treatment groups and treated for duration of 4 weeks.

Determination of sample size

According to the previous animal study, the difference between groups that we wish to detect is 30%. The statistical power is 0.90, the significance criterion is set to 0.05 and the two-tailed statistical analysis is considered, the formula used to calculate the sample size is as followed:

$$N = \frac{(\mu_\alpha + \mu_\beta)^2 4\pi(1-\pi)}{(\pi_1 - \pi_2)^2}$$

Where N is the total sample size,

π_1 = expected proportion in the treatment group;

π_2 = expected proportion in placebo group;

π is combined proportion of the two groups.

Tabulated value of μ_α=1.96

Tabulated value of μ_β=1.28

The total sample size is:

$$N = \frac{(1.96+1.28)^2 4 \times 0.35(1-0.35)}{(0.5-0.2)^2} = 106$$

As the drop out rate is about 15%, the total number of the study patients would have to be:

$$N = \frac{106}{1-0.15} = 126$$

So, the total number of subjects should be recruited for the trial is **126**, each group **63**.

Proper statistical methods for TCM data analysis

Many randomized trials involve measuring a continuous outcome at baseline and after treatment. There are four possibilities for clinical trials data analysis: 1) post-treatment; 2) change between baseline and post-treatment; 3) percentage change between baseline and 4) post-treatment and analysis of covariance (ANCOVA) with baseline score as a covariate.

One can use the baseline score solely to ensure baseline comparability and enter only the post-treatment score into analysis ("POST").

One can analyze the change from baseline, either by looking at absolute differences ("CHANGE") or a percentage change from baseline ("FRACTION").

Some trials assess outcome several times after treatment, the design known as "repeated measures." The most sophisticated method is to construct a regression model that adjusts the post-treatment score by the baseline score ("ANCOVA").

We use D&G clinical trial data as example to elucidate the importance of the proper

statistical method for a clinical trial. In the study, we wished to evaluate carotid intima–media complex thickness (IMT) as a marker of atherosclerosis to evaluate the cardiovascular-protective effects of Danshen-Gegen (D&G) treatment in high-risk hypertension subjects. When analyzing IMT data, if we used common student t-test to compare the difference between the two groups at each time point, we could found there were no differences at each visit. That mean the study medicine D&G had no effect on IMT. However, if we used ANCOVA to analyze the same data, the results were quite different, which demonstrated that D&G had effectiveness in decrease of IMT **(Table 7-6)**.

Table 7-6 Changes in IMT

Group	Baseline	6-month	12-month
D&G(2gm+1gm)	0.818±0.178	0.795±0.173	0.784±0.157
Placebo	0.797±0.178	0.802±0.173	0.811±0.169
p-value[1]	0.607	0.870	0.493
p-value[2]	0.607	0.037	0.001

p-value[1]: comparison between D&G(2gm+1gm) with placebo (Student *t-test*)

p-value[2]: comparison between D&G(2gm+1gm) with placebo (ANCOVA)

The reason for this inconsistency was that the initial conclusion was based on group mean that had not been adjusted for the effect of the baseline covariate.

The findings presented here reconfirm that ANCOVA is the method of choice for analyzing the results of trials with baseline and post treatment measurement.

In cases where ANCOVA cannot be used, such as with small samples or where the assumptions underlying ANCOVA modeling do not hold, CHANGE or POST are acceptable alternatives, especially baseline variables are comparable between groups (perhaps ensured by stratification) and if correlation between baseline and

post-treatment scores are either high (for CHANGE) or low (for POST).

For example in Osteoporosis clinical trial data analysis, we analyzed the CHANGE of BMD from baseline. The results indicated that subjects over 10 years after menopause showed a 0.7% increased after 12 months herbal treatment. In the placebo group, bone loss over 12 months reached 0.6% (p=0.067)(**Figure 7-10**).

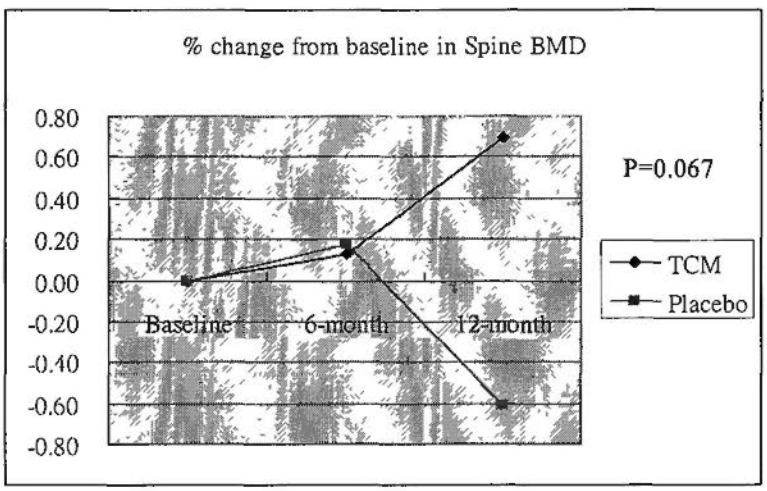


Figure 7-10 Changes in Spine BMD over 12-month treatment in the sub-group of menopausal duration > 10 yr

ANCOVA has the highest statistical power. Change from baseline has acceptable power when correlation between baseline and post-treatment scores is high; when correlation is low, analyzing only post-treatment scores has reasonable power. Percentage change from baseline has the lowest statistical power and was highly sensitive to changes in variance. Theoretical considerations suggest that percentage change from baseline will also fail to protect from bias in the case of baseline imbalance and will lead to an excess of trials with non-normally distributed outcome data.

If treatment assignment is based on the baseline, only ANCOVA is unbiased. In randomized studies and studies with treatment assignment depending on the baseline, ANCOVA must be used (28, 29).(**Table 7-7**)

Table 7-7 Statistical power of each method of analysis

Correlation	ρ = 0.2	ρ = 0.35	ρ = 0.5	ρ = 0.65	ρ = 0.8
POST	70.5%	70.5%	70.5%	70.5%	70.5%
FRACTION	45.1%	56.4%	67.0%	82.7%	97.1%
CHANGE	50.7%	59.2%	70.5%	84.8%	97.7%
ANCOVA	72.3%	76.1%	82.3%	90.8%	98.6%

Specific statistical tests that are associated with the different types of clinical outcome measures depending on whether outcome variables are categorical, continuous, or clinical event.

Categorical outcomes are often subdivided into nominal and ordinal types.

- Nominal outcomes are often dichotomous variables such as yes/no, male/female, or present/absent. These are categories that are not ordered (sex, blood type, presence of metastases). Death, as described previously, would also fall in to the categorical – nominal outcome type.
- Ordinal outcomes are categories that are ordered (degrees of pain, patient quality of life). These are usually represented by scales evaluated by either the patient or the physician.

Continuous outcomes quantify intervals on an infinite scale of values (tumor size, skin thickness).

Clinical-event outcomes are usually either the length of time after an intervention (treatment course, surgical excision, diagnosis) to the final predetermined outcome or the number of events during a fixed duration of follow-up (RM 295). This outcome is often used in trials of immunosuppressants for autoimmune skin disorders such as pemphigus for evaluating time until clinical relapse.

If the primary outcome variable is a clinical event, then it may be analyzed as a proportion for occurrence in a fixed time period (higher risk = greater incidence of the outcome variable at the end of the fixed period than at the beginning). The test for comparing proportions is the χ^2 test.

Table 7-8 Statistical tests commonly used in clinical trials

	Two groups of different subjects	Three or more groups of different subjects	Pre- and post-intervention in same subject	Two or more treatments in same subject
Categorical	X^2 test	X^2 test	McNemar's test	Cochrane Q-test
Continuous (normal distribution)	Unpaired t-test	ANOVA	Paired t-test	Repeated measures ANOVA
Continuous (non-normal distribution)	Mann-Whitney rank sum test	Kruskal-Walls statistic	Wilcoxon signed-rank test	Friedman statistic

In some cases, higher risk is reflected by an earlier occurrence of the event. This is known as survival analysis and is applied when patients are followed for varying lengths of time and at the end of follow-up, the patients may or may not have experienced the outcome variable (generating a Kaplan–Meier survival curve).

Statistical tests applied to data for two groups differ from those applied to three or more groups. For instance, the statistical test used for two groups with a continuous outcome variable is either the unpaired t-test or the Mann–Whitney rank sum test. Whereas the statistical test for three or more groups with a continuous outcome variable is the analysis of variance (ANOVA) test or the Kruskal–Wallis test.(**Table 7-8**)(30)

The outcome variable is normally distributed or not will determine whether a parametric or a nonparametric test should be used. Parametric tests are used for data that are sampled from a normal distribution (the sample is distributed in a bell-shaped

curve). Nonparametric tests are used when the data is not normally distributed (nonbellshaped curve). In general, clinical research data is not normally distributed and thus nonparametric statistical analysis is preferable. But because statistical significance is more difficult to show with nonparametric tests, most studies reported in the medical literature use parametric statistical methods.

Paired data involve one group of patients receiving two or more interventions, whereas unpaired data compares two or more different groups of patients receiving one intervention. For example, if an investigator measures the outcome in the same subject twice, these data are paired because they are measured in the same subject.

The sample size determination for the DBT clinical trial is dependent on the primary endpoint, which is frequency and severity of hot flushes. The sample size was calculated using data for hot flush frequency from many previous trials of Hormone Replacement Therapy (HRT), herbal medicine treatment and acupuncture treatment. In **stage I** trial, assuming similar efficacy to that of oestradiol in eliminating hot flushes (expect reduction in prevalence from 0.67 to 0.32), with alpha=0.05 and power 0.90 need 41 patients / group. Therefore, to allow for approximately 20% dropouts, 100 women were recruited for Stage I trial.

Interim analysis for TCM clinical trial

Clinical trials tend to have a long duration, because patients are usually recruited one by one, and especially subjects' responses to herbal medicine treatment are need relatively long time compared with chemical medicines (31). The utilization of interim analyses to compare treatment arms, terminate the development of ineffective or unsafe drugs, or accelerate the regulatory approval process of breakthrough drugs

has gained popularity in the pharmaceutical industry (32). As the sample-size of a clinical trial is generally based on preliminary and/or uncertain information, an interim check on the un-blinded data may be useful to reveal whether or not overall response variances, event rates were as anticipated.

As there were many outcome variables in the DBT clinical trials, but in the interim analyses only analyzed key endpoints. In the DBT study, we limited the number to only the major variables and conducted when half subjects completed the study.

An interim analysis is any assessment of data done during the patient enrollment or follow-up stages of a trial for the purpose of assessing center performance, the quality of the data collected, or treatment effects (33). Interim analysis is also called "data-dependent stopping" or "early stopping". Interim analyses are most often used to find convincing enough evidence to say that there is a significance large treatment difference, and that the difference is convincing enough to chance the trial at a point earlier than planned at first.

Ethical and economic reasons are also taken into consideration to stop the trial early (34). The ethical reason is the most important reason to stop the trial. We want to make sure that the maximum number of patients receives the most effective treatment at the earliest stage. Since clinical trials are expensive, there are also economic reasons to include as few patients as possible. Interim analysis is also used to possibly reduce the expected number of patients and to shorten the follow-up time needed to make a conclusion.

To avoid sample size under- or overestimation, an internal estimate of the variability may be used for the reassessment of sample size in the course of the study.

To calculate the sample size usually a variance estimate is taken from previous data.

However, there may be considerable differences in the environment (population, measurement procedures, treatment modalities, etc) which may influence the variability in the forthcoming trial. This -- in case of an underestimation of the variability in the planning phase -- can lead to a considerable loss of power.

Clinical trials tend to have a long duration, because mostly patients are enrolled one by one, and their responses to treatment are observed sequentially.

In clinical trials of efficacy, data are accumulated sequentially from observations on subjects who are entered one at a time. Accrual of subjects ends when the predetermined sample size has been reached. Then the data can be analyzed to test the null hypothesis of no difference in outcome between the experimental and control treatments. However, as the trial proceeds, the investigator, in addition to addressing the ethical and financial aspects of the trial, has the responsibility of monitoring the response variables to ensure the scientific results are precise, so that any dramatic benefits or potentially harmful effects can be identified, as soon as the evidence becomes clear. An interim analysis is undertaken primarily to identify whether a point has been reached in the trial, beyond which it is unethical to continue recruiting subjects, who may receive a treatment known to be inferior.

We take DBT clinical trial as example to demonstrate the interim analysis.

The background

Title: A Randomized, Double-Blind, Multiple-Dose Escalation Study of the Effect of *Danggui Buxue Tang* (当归补血汤) on symptomatic postmenopausal Hong Kong Chinese Women

Dosage design:

Low dose: take DBT 1.5g daily for 3 months

Middle dose: take DBT 3g daily for 3 months

High dose: take DBT 6g daily for 3 months

The interim analysis

Primary Objective

To examine optimal dose of *Danggui Buxue Tang* (当归补血汤) in treatment of menopausal symptoms of hot flushes and sweating

To evaluate the safety and tolerability of *Danggui Buxue Tang* (当归补血汤) in patients with menopausal symptoms

Secondary Objective

To evaluate the effect of *Danggui Buxue Tang* on quality of life of patients with menopausal symptoms

Parameters

This interim report is analyzing the following parameters:

1 MENQOL--Vasomotor domain

- Hot Flushes
- Night Sweats
- Sweating

2 Greene Climacteric Scale—Vasomotor cluster

- Hot Flushes
- Night Sweating

3 Daily record—Self-reported symptoms

- Number of hot flushes

Mild hot flushes

Moderate hot flushes

Severe hot flushes

- Number of night sweats

Statistical methods for Interim analysis

All analyses for baseline characteristics were performed by using one-way analysis of variance (ANOVA)..

Analysis of covariance (ANCOVA) has the highest statistical power. ANCOVA is the method of choice for analyzing the results of trials with baseline and post treatment measurement. Comparisons among the three groups during and post- DBT treatment period were performed using analysis of covariance (ANCOVA, with baseline as a covariate).

Analysis of within-group differences from baseline was to use the paired t test.

$P<0.05$ was considered statistically significant.

The statistical analyses were made with SPSS 14.0 for windows.

Interim analysis Results

Baseline information

Total 23 subjects entered this interim analysis, 8 subjects in high dose group, 8 subjects in middle and 7 subjects low dose group.

As shown in **Table 7-13**, the baseline characteristics were comparable. There were no statistical differences among the three groups.

Table 7-9 Baseline characteristics

	High dose n=8	Middle dose n=8	Low dose n=7	P value
Vasomotor domain				
Hot Flushes	4.25±0.71	4.63±1.41	4.57±1.40	0.802
Night Sweats	3.50±1.07	3.80±1.64	3.67±1.51	0.924
Sweating	3.88±1.36	3.63±1.41	4.50±1.38	0.505
Greene Climacteric Scale				
Hot Flushes	2.88±0.64	3.25±0.71	3.43±0.53	0.247
Night Sweating	2.63±0.52	2.25±1.04	2.71±0.76	0.494
Daily Records				
No. of mild hot flushes	1.84±1.44	2.63±2.26	1.61±1.31	0.496
No. of moderate hot flushes	2.86±1.96	3.00±2.20	1.49±0.92	0.238
No. of severe hot flushes	0.91±1.27	2.21±3.16	1.49±1.88	0.528
No. of night sweats	1.42±1.22	1.39±2.20	0.88±1.12	0.778

Total 23 subjects entered this interim analysis, 8 subjects in high dose group, 8 subjects in middle and 7 subjects low dose group.

As shown in **Table 7-9**, the baseline characteristics were compatible. There were no statistical differences among the three groups.

Table 7-10 Vasomotor domain

Dosage	Screening	Visit 1	Visit 2	Visit 3	Visit 4	P value (pre : post)
Hot Flushes						
High(H)	4.25±0.71	4.00±0.93	3.00±1.60	3.40±0.55	2.40±0.89 (-44%)	0.005
Middle(M)	4.63±1.41	4.00±1.07	3.50±1.60	3.29±0.95	2.14±0.69 (-54%)	0.002
Low(L)	4.57±1.40	4.43±1.13	3.86±1.35	3.71±1.70	3.43±1.51 (-25%)	0.047
P value	0.802	0.591	0.656	0.807	M:L 0.078	
Night Sweats						
High	3.50±1.07	3.43±1.51	2.29±1.38	2.80±1.10	2.00±1.00 (-43%)	0.009
Middle	3.80±1.64	2.75±1.83	3.00±2.10	3.33±1.53	2.00±1.00 (-47%)	0.130
Low	3.67±1.51	4.50±1.73	3.60±1.67	3.20±1.79	3.20±1.64 (-13%)	0.208
P value	0.924	0.344	0.145	0.571	0.117	
Sweating						
High(H)	3.88±1.36	3.50±1.69	2.57±1.81	3.75±0.50	2.40±0.89 (-38%)	0.035

Middle(M)	3.63±1.41	3.00±1.85	2.75±1.83	2.29±0.95	2.50±0.71 (-31%)	0.295
Low(L)	4.50±1.38	5.00±1.26	4.00±1.55	4.33±1.51	3.67±1.75 (-18%)	0.289
P value	0.505	0.204	0.444	M:L 0.033	0.392	

As shown in **Table 7-10**, hot flushes of MENQOL in all dose groups were remarkably improved after DBT treatment when compared with their baseline. In high dose group the scores of hot flushes were decreased 44% (p=0.005); in middle dose group the scores decreased 54% (p=0.002), while in low dose group the scores only decreased 25% (p=0.047).

At the end of study (visit 4), there were no statistical difference in hot flushes between high dose group and middle dose group (p=0.869) and also no difference between high dose and low dose group (p=0.872). However, between the middle and low dose group the difference was very close to the statistically significant level (p=0.078). **(Table 7-10, Figure 7-10)**

At the end of study (visit 4), there were no significant differences in night sweats between these three dose groups (p=0.117) (**Table 7-10, Figure 7-11**).

When compared with baseline the scores of Night sweats in high dose group were decreased 43% (p=0.009); middle dose group decreased 47% (p=0.130), low dose group decreased 13% (p=0.208). **(Table 7-10, Figure 7-11)**

At the end of DBT treatment (visit 3), there were only statistical difference in the scores of sweating between middle and low dose groups (p=0.033), no differences between high dose and low dose group (p=1.000). **(Table 7-10)**

When compared with baseline, the scores of sweating in high dose group was decreased 38% (p=0.035), middle dose group decreased 31% (p=0.295) and low dose group decreased 18% (p=0.289). **(Table 7-14, Figure 7-12)**

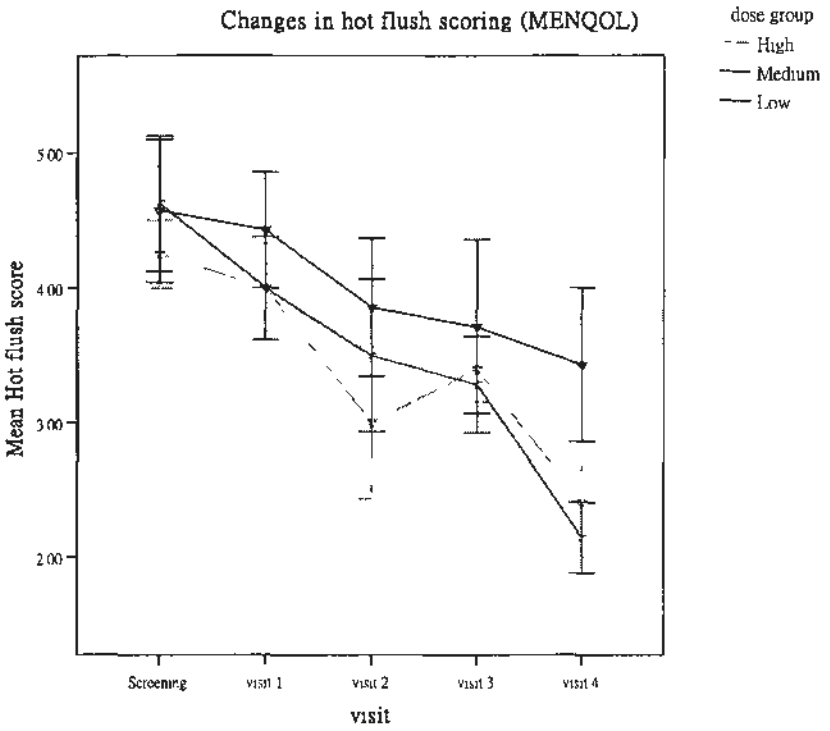


Figure 7-11 Changes in hot flush scoring (MENQOL)

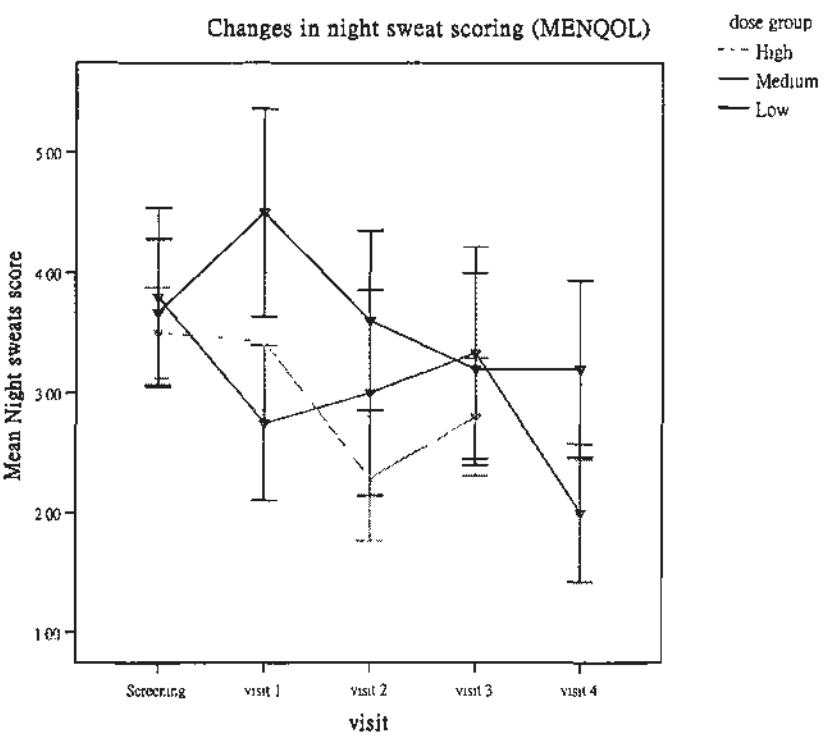


Figure 7-12 Changes in night sweat scoring (MENQOL)

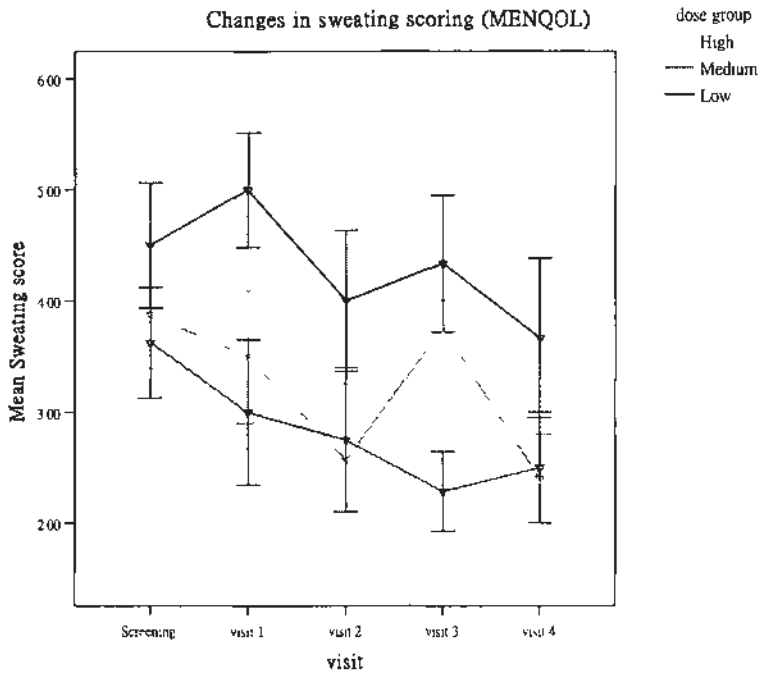


Figure 7-13 Changes in sweating scoring (MENQOL)

Greene Climacteric Scale

Table 7-11 Greene climacteric scale

Dosage	Screening	Visit 1	Visit 2	Visit 3	Visit 4	P value (pre : post)
Hot Flushes						v3/v4
High	2.88±0.64	3.25±0.46	2.88±0.64	2.29±0.95	2.40±0.55 (-17%)	0.374
Middle	3.25±0.71	3.25±0.46	3.00±0.76	2.50±0.76	2.13±0.35 (-34%)	0.002
Low	3.43±0.53	3.43±0.53	3.29±0.49	3.14±0.69	3.29±0.76 (-4%)	0.356
P value	0.247	0.746	0.805	0.287	M:L 0.003	
Night Sweating						
High	2.63±0.52	2.75±0.71	2.25±0.71	2.14±0.90	2.40±0.55 (-9%)	0.374
Middle	2.25±1.04	2.63±0.74	2.38±1.06	1.75±0.89	1.50±0.76 (-33%)	0.003
Low	2.71±0.76	2.43±1.13	2.57±1.27	2.43±1.27	2.14±1.07 (-21%)	0.030
P value	0.494	0.362	0.310	0.725	0.121	

Hot flushes of Greene Climacteric scale in Middle dose group were significantly

improved at visit 4 of post-treatment when compared with other dose groups (p=0.003) and the baseline (p=0.002). No changes were observed in high and low dose groups. (**Table 7-11**, **Figure 7-13**)

Night sweating scores in middle and low dose groups were improved when compared with their baseline (p=0.003 and 0.030). (**Figure 7-14**)

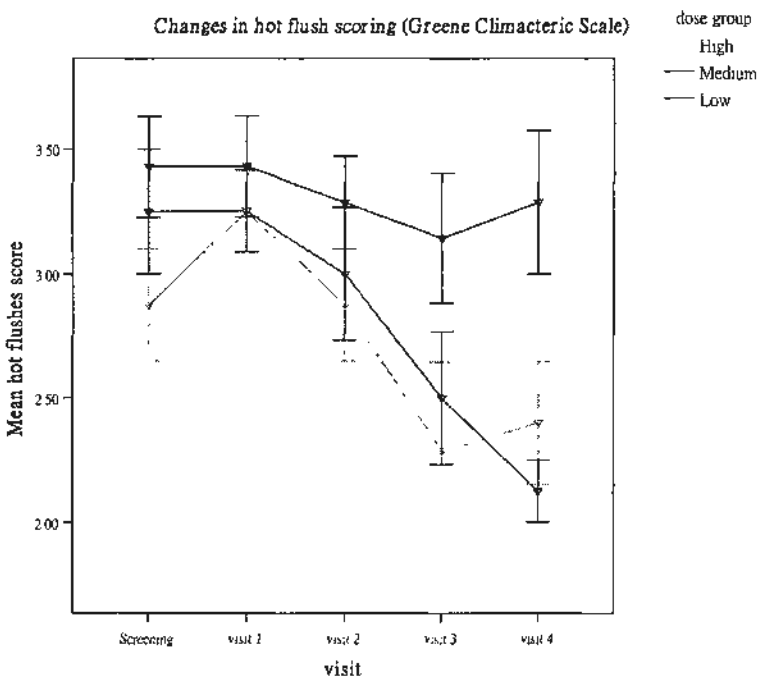


Figure 7-14 Changes in hot flush scoring (Greene Climacteric Scale)

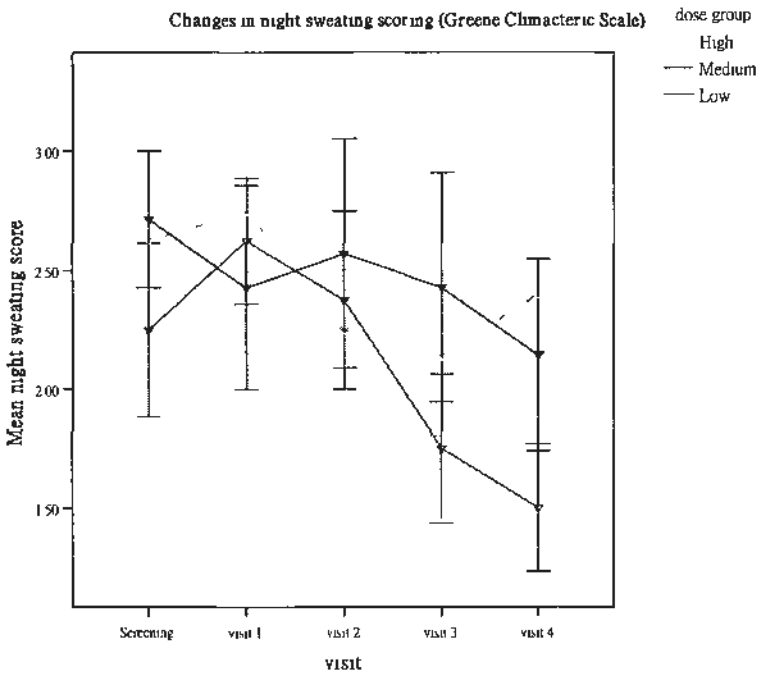


Figure 7-15 Changes in night sweating scoring (Greene Climacteric Scale)

Daily records—average number of hot flushes and night sweats

Table 7-12 Daily records—hot flushes and night sweats

	Visit 0	Visit 1	Visit 2	Visit 3	P value (pre : post)
Hot Flushes					
Mild					
High dose	1.84±1.44	1.71±0.62	1.64±1.07	1.38±1.40 (-25%)	0.787
Middle dose	2.63±2.26	2.70±2.33	2.15±2.32	1.93±2.45 (-27%)	0.088
Low dose	1.61±1.31	1.25±1.44	1.83±1.42	1.39±1.99 (-14%)	0.548
P value	0.496	0 477	0 872	0.995	
Moderate					
High dose	2.86±1.96	2.04±2.06	1.51±1.73	1.82±2.00 (-36%)	0.088
Middle dose	3.00±2.20	2.88±2.52	2.15±2.05	1.24±2.05 (-59%)	0.001
Low dose	1.49±0.92	2.11±1.07	0.89±0.87	0.64±0.46 (-57%)	0.011
P value	0.238	0.422	0 576	0.615	
Severe					
High dose	0.91±1.27	0.70±1.14	0.35±0.92	0.38±0 80 (-58%)	0 259
Middle dose	2.21±3.16	2.05±3.12	1.51±2.39	1.03±2.39 (-53%)	0.016

Low dose	1.49±1.88	1.31±1.27	1.32±2.01	0.81±1.37 (-46%)	0.031
P value	0.528	0.810	0.543	0.296	
Night sweats					
High dose	1.42±1.22	1.39±1.92	1.53±1.71	1.70±1.74 (+20%)	0.703
Middle dose	1.39±2.20	1.16±1.87	0.68±1.45	0.58±1.53 (-58%)	0.023
Low dose	0.88±1.12	0.86±1.40	0.85±1.24	0.67±1.05 (-24%)	0.017
P value	0.778	0.771	0.076	M:H 0.075	

Self-reported daily record data shown, the number of moderate and severe hot flushes and night sweats in middle and low dose groups was significantly improved when compared with baseline ($p<0.05$). (**Table 8-12**, **Figure 8-15, 8-16, 8-17** and **8-18**)

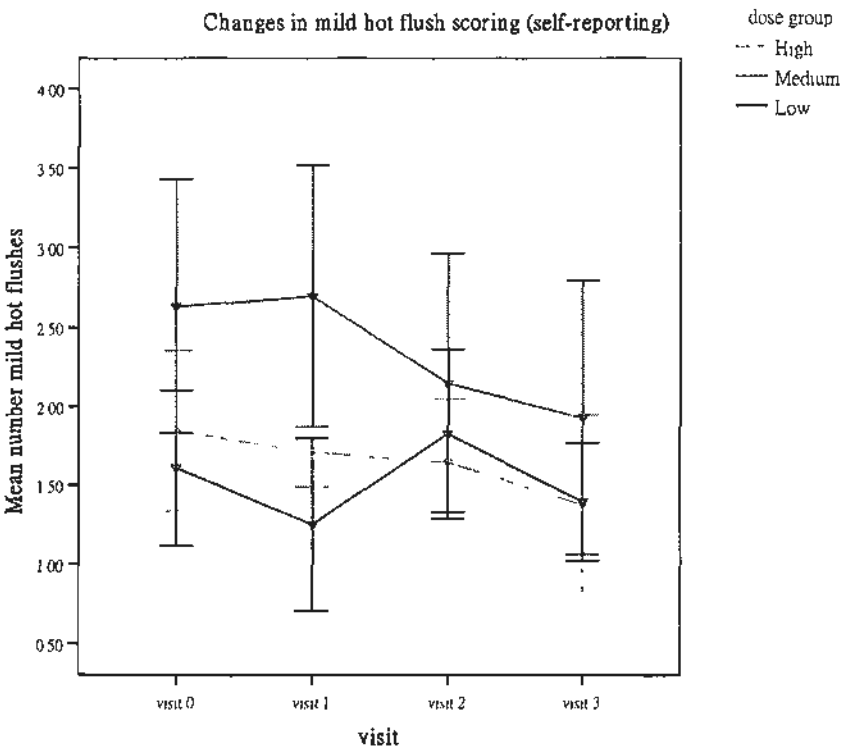


Figure 7-16 Changes in mild hot flush scoring (self-reporting)

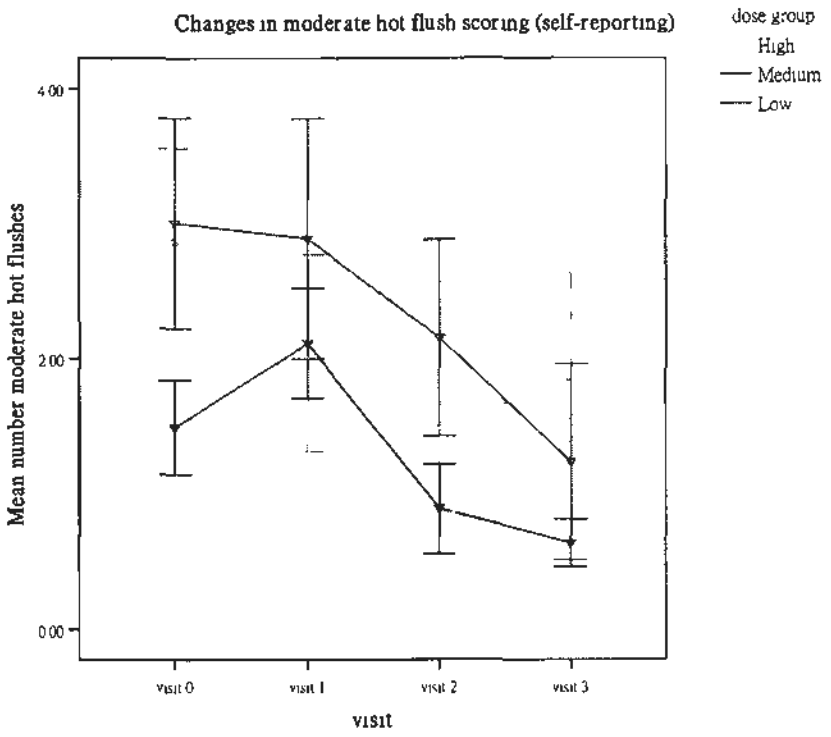


Figure 7-17 Changes in moderate hot flush scoring (self-reporting)

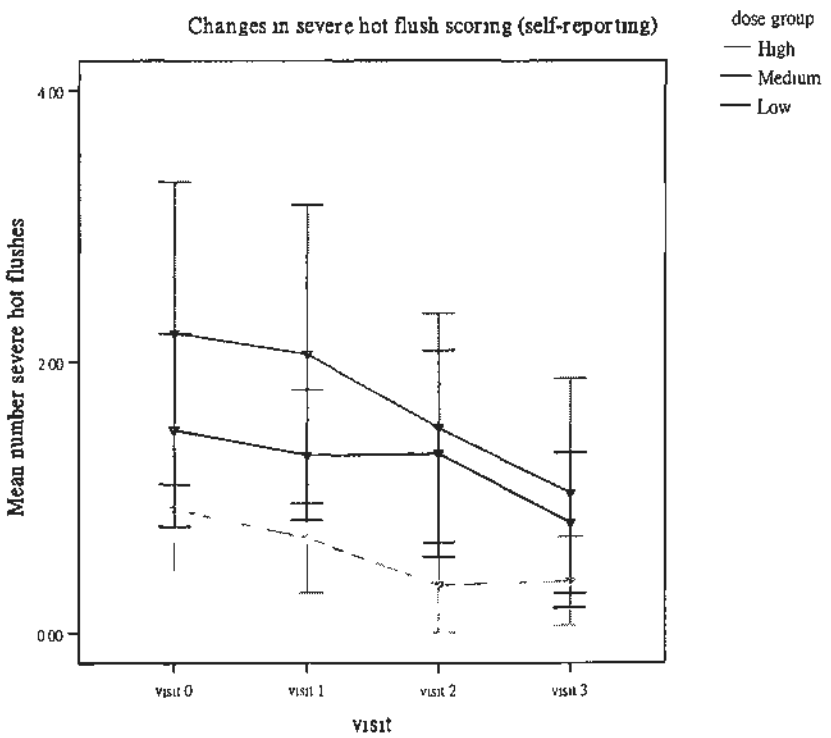


Figure 7-18 Changes in severe hot flush scoring (self-reporting)

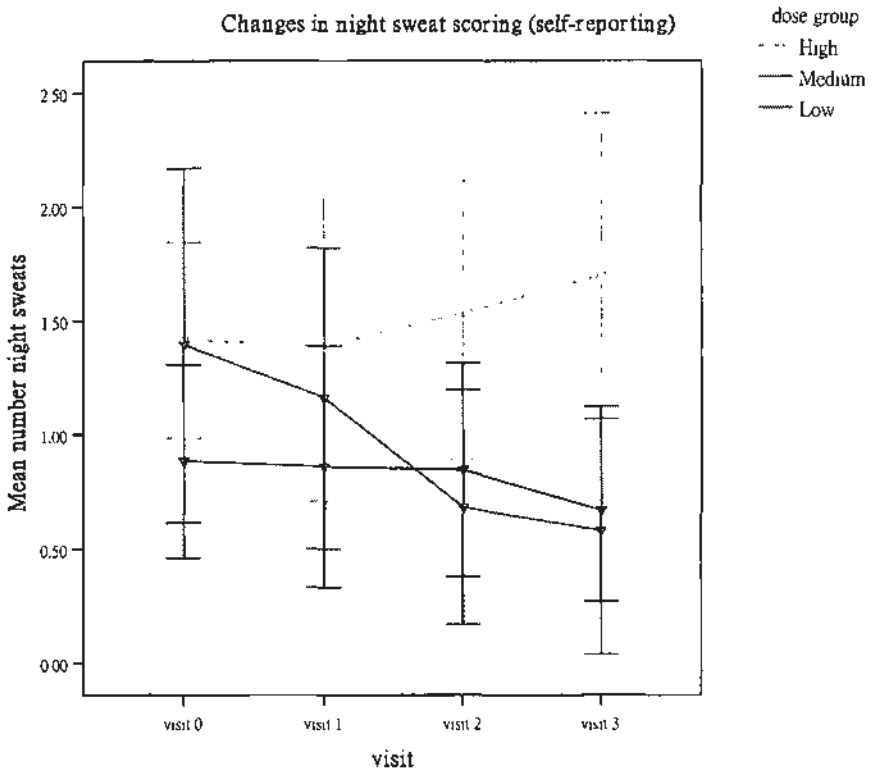


Figure 7-19 Changes in night sweat scoring (self-reporting)

Conclusion

1 MENQOL (Vasomotor domain), Greene Climacteric Scale scores (vasomotor cluster) and Self-reported symptoms were significantly improved after DBT treatment.

2 Among the three dosages, middle dose shown more effective than the other two dosages. It seems that the middle dose is optimal dose.

Sample size adjudged based on interim analysis results (example: atopic dermatitis stage III trial)

The future analysis

- Continue follow-up and update of information of the patients included in the trial.
- Additional analysis of the compliance and response to DBT will also be performed

The utilization of interim analyses to compare treatment arms, terminate the development of ineffective or unsafe drugs, or accelerate the regulatory approval process of breakthrough drugs has gained popularity in the pharmaceutical industry (35).

Interim analyses are most useful in late phase II or phase III trials. The assumptions made at the design stage involve a great deal of uncertainty. Thus, the safety profile of the drug and its efficacy and optimal dosing regimen may not be well characterized. Under such circumstances it is imperative to monitor the trial as the data accumulate so as to prevent the exposure of patients to unreasonable risks or impose on them the burdens of a clinical trial without having a reasonable expectation that the trial would produce useful information. Similarly, the uncertainty about the drug's efficacy may result in a trial designed to accrue many more patients than are needed to demonstrate its therapeutic activity. An interim analysis could result in the saving of patient resources and the shortening of the drug development and approval time. The clinical trial literature has demonstrated that interim analyses often result in important modifications of the trial conduct including early termination (36, 37).

Interim analysis typically involves unblinding the patients' treatment assignment before all the trial data have been collected. Masking the treatment is an essential mechanism of bias prevention. Thus, controlling the dissemination of preliminary results during an ongoing trial is a critical element of an interim analysis plan.

Interim analyses could make an invaluable contribution to the successful end of a trial,

even when "success" may mean a decision to stop the development of the drug. However, an interim analysis does have its risks and must be planned deliberately with attention to relevant issues.

The objectives of the interim analysis were to evaluate the overall safety of the drug, to examine the adequacy of the assumptions made at the design phase that led to the determination of the sample size, to decide whether early termination of the trial based on clear evidence of efficacy or lack thereof was warranted, and to determine whether there were other unanticipated reasons that might lead to a recommendation to terminate the trial early or modify the conduct of the trial.

The utilization of interim analyses to compare treatment arms, terminate the development of ineffective or unsafe drugs, or accelerate the regulatory approval process of breakthrough drugs has gained popularity in the pharmaceutical industry (35).

7.5 Case Report Form (CRF) preparation

Clinical data were reported on case report forms (CRFs). The Case Report Form (CRF) is a set of forms for each subject, provides a record of data generated according to protocol. These forms were to be completed on an ongoing basis during the study. During the study, the CRF was monitored for completeness, accuracy, legibility and attention to detail. The CRF was retained for review.

Following is a contents of CRFs which were used in DBT trial: Participant screening form, inclusion/exclusion criteria, medical history, physical examination, laboratory data- hematology, laboratory data – chemistry screen, laboratory data – hormone levels, drug compliance, concomitant medications, adverse event, serious adverse events, drop-out form, menopausal specific quality of life (MENQOL), patient diary of menopausal syndromes, and medication log.

Compliance was monitored via a drug compliance recorded on each visit of the study. Missing data was obtained via the data audit procedures and by the data entry system to the database.

7.6 Subjects recruitment and compliance–Strategies and challenges

The randomized controlled trial (RCT) is widely accepted as the most powerful research method in the evaluation of the efficacy of a study medication (38). These principles are also applied to traditional Chinese medicine (TCM) studies (39). Efficient and effective recruitment of patients and retention of participants are essential steps in the clinical trials. However, it has been shown that the most eligible patients do not participate in clinical trials. A study reported that in eligible patients who were aware of an appropriate clinical trial, 71% choose not to participate (40). In some RCTs, the dropout rate was as high as 80% (41). The results of recruitment are generally influenced by a number of factors, including the nature of the study itself, the recruitment strategy used, and the target population (42). In this paper, we examine some particular barriers to patient participation and explore possible solutions.

TCM and herbal medications have remained very popular in Chinese societies around the world because it has developed within a culture that has endured for more than 3000 years over a vast geographical area with an enormous population. Centuries of empirical studies and accumulated experience has retained good and useful medical practices while what was not effective was discarded. There still remains a high incidence of side effect and toxicity as well as TCM not providing expected benefits. Nowadays, when modern medicine fails to provide the expected benefits, TCM could be considered as a last resort; so an unrealistic perspective often exists in participants. It may therefore be easier to conduct TCM clinical trials in the Chinese community;

however, barriers or difficulties still exist.

Process of recruitment

It is important to define clearly the target population to be studied, which depends on the study protocol. The difficulty of recruitment depends on the exclusion/inclusion criteria, age, study medication, disease risk factor, biomarkers tested, stage of disease, etc. In our TCM clinical trials, the target populations are usually those with chronic diseases or with their conditions under control. Modern medicine has failed to obtain perfect solutions for chronic diseases or conditions that include allergic conditions, autoimmune diseases, cancers, chronic pain, chronic derangements, degenerative diseases, nerve damages, viral infections, etc. Our experiences tell us these patients would be willing to participate in TCM clinical trials because modern medicine is unable to help them. There are four stages in the actual recruitment process, which are clearly dynamic and interactive (43). These stages include:

1). Introducing the project to the potential patients

2). Explaining how to implement the project

3). Assessing and intensifying the understanding

4). Facilitating patients' decision-making.

The process of screening started at the time of initial contact with the study team, either in person or over the telephone. The initial approach is considered vital. It is essential that someone is assigned to telephone duty to deal with initial contact to avoid loss of potential volunteers. The primary purpose is to put the patient at ease and to establish rapport between patient and research team quickly. Table 1 lists our means that we usually use in our clinical trial center during the initial approach stage. When subjects called in response to the initial approach, the research nurse noted down their names, a contact telephone number to start screening on related eligibility

criteria, and whether they were using an herbal preparation. If a candidate who was using any herbal preparation was willing to participate the trial, our research nurse would advise the candidate to stop the herbal preparation for a suitable period (washout period) according to the requirement of the study protocol before they are enrolled. If the telephone screening criteria were met, the research nurse would arrange a proper interview for informed consent. Participants then progressed to laboratory testing for necessary function assessments. If the inclusion/exclusion criteria were satisfied and informed consent forms were obtained, the participants progressed to randomization to either TCM treatment group or placebo group.

Barriers and strategies of recruitment

Explicit inclusion/exclusion criteria are essential elements of any clinical studies. According to the trial protocol, potential subjects for TCM trial had to satisfy the inclusion/exclusion criteria. Their participation was quite different from visiting their general practitioner. If the inclusion/exclusion criteria of TCM clinical trials were too rigorous, the difficulty of recruitment is increased. For example, we had conducted a RCT by using a Chinese herbal formula named DBT to control post-menopausal symptoms. The protocol required that subjects should have at least 14 hot flushes per week and had not undergone any treatment including hormone therapy and herbal treatment, before they entered the study and during the study period. We initially approached 452 candidates, after screening, only 60 subjects were eligible, finally only 52 subjects completed the clinical trial (**Table 7-13**). Sometimes, we needed to identify particular Chinese herbs which were consumed by participants themselves to judge if there were any interaction with the study TCM medication. In this case, we advised the subjects to stop using these herbs before they were enrolled and during study period. However, many candidates were unwilling to do so. To deal with this problem, we advised the candidate keep using the herbal preparation during study

period, if our TCM practitioner found that the herbal preparation would not interfere with study herbal medication. To minimize the outcome bias, we treated this case very carefully. If we could not ensure whether the herbal preparation taken by the candidate had any interaction with the study herbal medication, and the candidate was unwilling to stop taking his/her herbal preparation, we had to exclude the candidate.

The recruitment procedures are not straightforward. To enhance recruitment through television interviews, newspaper releases, and advertisements to make use of media would be very useful. However, to arrange screening interviews with patients and retaining them in the trial without drop out was even more difficult.

Liaising with local organizations and collaborating with local practitioners would be helpful for recruitment, especially when nonprofit organizations were involved. Referral from physicians was another channel of recruitment, but it had not been a main channel in Hong Kong. In our experience, recruiting patients through the health services (hospital consultants, general practitioners, and clinics) is likely to be less successful because professional heath specialists had other priorities.

Table 7-13 Patient recruitment for a traditional Chinese medicine formula trial

Process of recruitment	**Case**
Initial approach	452
Assessed for eligibility	71
Screen failures	11
Enrolled / Randomized	60
Drop out	9
Completed	52

In general, the means of initial contact with potential volunteers that we targeted in our TCM clinical trials included advertisements and relevant articles in newspapers, public seminars, television interviews, announcements in community centers, etc **(Table 7-14)**.

Table 7-14 Initial approach to patients

Initial approach	**Our experience**
Advertisement (newspapers)	TCM for Postmenopausal symptoms
Public Seminars	Herbal formula for lung and colon cancer
Published articles (usually in newspapers)	TCM for Postmenopausal symptoms
Television interview	TCM and postmenopausal symptoms
Physician referral	TCM for diabetic foot ulcer
Outpatient/in-house physician referral	TCM for influenza prevention
Friend or acquaintance referral	TCM for influenza prevention
Non-Profit and non-government Organizations (NGO)	Herbal formula for lung and colon cancer
Community (letters and posters)	Acupuncture for smoking cessation, osteoporosis study
University collaboration	TCM formulas for post-stroke and insomnia

Appeals in newsletters may also be helpful but not as the primary channel for recruitment. According to our experience, advertising in local newspapers and announcements in community centers are effective. But an optimal approach should depend on the nature of the study. For example, announcements in community centers were efficient for non-specific diseases/conditions such as a smoking cessation trial and menopausal symptoms. Considering the main channel of initial approach it should depend on the nature of study disease/condition. For the diseases/symptoms eg, postmenopausal symptoms, that the public have been aware of and with a higher prevalence, the initial approach through advertisement would be more efficient. For those that were specific or rare in prevalence, eg, diabetic foot ulcer, a referral by physicians was the best way. For most diseases/conditions, multiple approaches were needed.

Poor clinical trial recruitment and retention impede the successful evaluation of new and existing interventions, and delay processes of clinical implementation (44). Patients may not want to take a chance with randomization in an environment where

the patients' enthusiasm is often in favor of TCM and against receiving a control treatment or no treatment. Another cause of low recruitment especially for TCM trial is the dosage form and taste of the study herbal preparations. Dosage forms of TCM usually include capsules, tablets, pills, sachets, and oral liquids. If the study drug was prepared in oral liquid and the taste was not good, and a large amount of doses was required to consume, the difficulty of recruitment is increased. We used to have a TCM clinical trial which required the participants to take up to 12 TCM capsules daily for 6 months. We found the difficulty in recruitment increased significantly and compliance decreased.

Besides, many candidates were worried about whether the study herbal preparation could change their body nature, for example, let them become hot or cold. If they believed some ingredients in the formula could change their body nature, they might refuse to participate in the trial. To reduce their worries, we had a TCM practitioner to provide consultation to the participants during study period.

Some candidates wondered if certain foods should be avoided during taking the study herbal preparation. It would be inconvenient for participants if some herbal soups are to be avoided for several months. This was certainly a barrier for recruitment. Another barrier was the study duration. The efficacy of TCM was usually believed to be slow, which made the duration of the clinical trial longer. This keeps the subjects stay with the clinical trial a quite long time, and certainly increased the difficulty of recruitment and retention. Furthermore, long duration of trial might lead to delay of proper treatment, or worsening of the disease.

Randomized controlled trials of new treatment often include a control group receiving no treatment or placebo. However, as patients often assume that a new TCM treatment is likely to be effective, recruitment to trials with a placebo arm is more difficult than recruitment to those of active treatment (45). No one liked to be assigned to take

placebo or go without treatment for a long-term period; they believed that they are likely to be exposed to a higher risk of disease deterioration compared with standard treatment. Therefore, greater reluctance from physicians and patients to participate in such designed study are expected (46, 47). Some subjects were willing to participate in the trial but did not want to discontinue their concomitant complementary or alternative medicines, which they firmly believe would support their good health. So willingness to enter a randomized controlled trial of TCM would be lower when the trial included a placebo arm.

The barriers to recruitment also came from fear, distrust, or misunderstanding of the clinical trial process, unwilling to take herbal medication, unacceptable to some ingredient(s) of study TCM formula or they would not be treated like a "guinea pig". We conducted a randomized placebo controlled double blind clinical trial to investigate the efficacy of *Coriolus Versicolor* (*Yunzhi*) in breast cancer treatment. In this study, nearly 98% subjects were already taking other herbal drugs. They were reluctant to be randomized for fear of inclusion into the placebo group. Eventually, we had to compromise and change the inclusion criteria, allowed those taking herbal products to participate as long as they stick to use their herbal product without change during study period. From the point of view of clinical trial, this arrangement certainly caused bias; however these subjects were cancer patients, we had to balance ethics and research.

For some patients, the barriers would come from time constraints, travel problems, worry about overdoing the blood tests, too many visits scheduled, instead of an active medicine.

For clinicians, the main barriers come from too few patients being referred to the trial, too complicated outcome measurements, too little time to devote to recruitment activities, and too few patients having the disease or problem being tested. Insufficient

recruitment was also due to Western doctors' unsatisfactory attitude to TCM study.

In Hong Kong, because of historical reasons, Western medicine is the official and mainstream healthcare provision. Western doctors trained in modern medicine have fewer opportunities to be trained in TCM. As they do not understand TCM well, they usually don't want their patients to take TCM before and during conventional treatment. This forms a barrier between modern medicine and TCM in Hong Kong. As a result, fewer candidates were referred to TCM clinical trials by Western doctors. For example, when we conducted cancer clinical trials using TCM as adjuvant treatment, we often had difficulties in patient recruitment because Western doctors did not encourage their patients to take TCM during conventional treatment.

There may be many clinical trials competing for these few patients who are willing to participate. Many physicians in Hong Kong usually do not encourage their patients to consider clinical trial participation, especially TCM clinical trials. These certainly increase the difficulties in subject recruitment for TCM clinical trials.

Table 7-15 summarizes the difficulties in recruitment.

Table 7-15 Reasons for recruitment barriers

	Reasons for recruitment failure	**Occurred in our TCM trials**
1	Unwilling to take herbal medication	In clinical trial "Herbal Formulae KGL (抗感靈) for Prevention and Treatment of Several Acute Respiratory Syndrome", some health volunteers were unwilling to take study herbal preparation because of the taste.
2	Lack of interest in participating	In Danggui Buxue Tang (當歸補血湯) on symptomatic postmenopausal trial and KGL (抗感靈) for prevention and treatment of several acute respiratory syndrome trial, many candidates we approached had no interest in clinical trials
3	History of renal or liver diseases, or active GI diseases	Some candidates who were eager to participate in TCM trial were ineligible because of their renal and liver functions were unsatisfactory to the inclusion criteria, e.g. diabetic foot ulcer trial, lung cancer trial and colorectal cancer trial
4	Out of the age range	In asthma clinical trial, we had to exclude some candidates because they were out of the age range.
5	Participating in conflicting study	Some candidates could not be recruited in Danggui Buxue Tang (當歸補血湯) trial as they were participating in other trial of hormone replacement therapy
6	Unwilling to commit to required time	Some health volunteers or candidates whose problems were not severe, were unwilling to follow the scheduled time, e. g.

		Danggui Buxue Tang (當歸補血湯) and KGL (抗感靈) trial.
7	Not willing to be randomized to non-treatment or placebo group	In the trial of Yunzhi & Danshen on immunological function in breast cancer patients, nearly all subjects were unwilling to take placebo.
8	Unacceptable to some ingredient(s) of the TCM formula	Due to intolerance of Danggui, some subjects refused to take part in Danggui Buxue Tang (當歸補血湯) trial
9	Refusal to traumatizing assessment, e. g. frequent blood taking	Some health volunteers refused to participate in KGL (抗感靈) trial as frequent blood taking was required.
10	Patient with significant medical problem e. g. allergy	Some candidates were excluded as they had history of hypersensitivity to drugs (including herbal drugs), e.g. asthma and allergic rhinitis clinical trials
11	Unable to comply with the protocol requirements e. g. arranged visits, appointed visit site etc.	Some candidates were unable to comply with the scheduled visits because of working or trips, e.g. in colorectal cancer trial some subjects had to work and some wanted to travel.

When insufficient recruitment occurred, we often consider the following steps which included reevaluating the required sample size; adding new sites to the trial; frequency of visits; extending the patient recruitment period, and modifying the patient inclusion/exclusion criteria. McDonald and colleagues reported that in about 10% of trials the inclusion criteria were changed or protocol amended to improve recruitment. (48)

Attempts to enhance the recruitment the following strategies were tried:

a) face-to-face contact with patients (eg, presentations at cancer support group);

b) post contact at selected work sites (eg, flyers and posters distributed at community);

c) notices in mass media (newspapers, journals, and broadcast media).

In addition, the research team encouraged the participants to notify their friends and family members who might be eligible for enrollment. Posters and flyers were delivered to related medical clinics and nonprofit making and non-government organizations.

It was important to involve a registered TCM practitioner in a TCM clinical trial in order to keep the subjects clear from other herbal medicine/functional foods, with vague precise defi nition, and keep the subjects without withdrawal. The role of the

TCM practitioner was to explain which herbal preparations or functional foods are to be avoided and provide consultation to the subjects while they were taking study medication. But no individualized prescription was given. This arrangement strengthened the relationship between subjects and investigators and the retention was much improved.

Recruitment to trials might be increased if information given to potential participants was sufficient including the potential personal benefits and risks.

Frequent contact with subjects is essential for maintaining volunteer motivation to keep them stay in the study. We had a TCM practitioner involved in the trial to interpret the individual herb properties of the study herbal formulation and answer the questions raised by subjects during study period. This arrangement worked well in subject retention.

Possible solutions

Proper evaluating inclusion criteria

Inclusion criteria that are too strict may bar many subjects from entering the study. We used to exclude all subjects who were taking herbal preparation as dietary supplements. These strict inclusion criteria made the recruitment difficult because about 50% to 60% of the population in Hong Kong has consulted TCM practitioners (49). According to a survey conducted by Critchley and colleagues (50), in 259 surveyed adult Chinese patients admitted to a Hong Kong teaching hospital, 90% used Chinese herbs on a regular daily basis in traditional soups and teas while 44% had consulted a TCM practitioner in the last twelve months prior to admission, but mainly for health promotion (59%) and minor ailments (30%). Only 25% sought advice for their current illness and 13% were taking regular traditional Chinese medicines prior to admission. The ingredients were difficult to identify. The use of Chinese herbs in

Chinese communities is very popular. If we exclude the subjects who take herbal preparations as dietary supplement or as medication, we would have great difficulties. To balance the recruitment accrual and result objectivity, we should encourage the eligible subject to stop taking other herbal preparations before and during study period. However, if the subject is not willing to quit, we had to advise the patient to just use the same herbal preparation at the same dosage throughout the study period. It would be useful for later data analysis if we had accurate information about the exact herbs being consumed.

Improving the quality of interactions with patients

All staff that interacted with patients during the recruitment process should be evaluated for the appropriateness and professionalism of their practice. This includes enthusiasm for the project, willingness to accept extra duties, empathy for the patient, and ability to discuss clearly the values and limitations of the trial. Experienced patients could be asked to share their experiences. We believe that the collaboration between research nurse and TCM practitioner in the process of patient recruitment and retention would significantly improve the efficiency and effect.

Shifting placebo-controlled to active controlled arrangements if possible

A meta-analysis of dropout rate comparison in the active treatment and placebo treatment revealed that use of a placebo-controlled design had a major effect on the dropout rates (41, 48). The dropout rates may be higher in the placebo group than in the active group because of the lack of efficacy of placebo, which caused patients to leave the study prematurely. In recent years, ethical objections have been raised against the use of a placebo "treatment" once an efficacious standard treatment has been established. To replace the placebo-controlled arm with an active-controlled arm

will be very helpful in patient retention if this change does not influence the outcome of the clinical trial.

Interest and motivation

Our patient population would have little incentive to participate in a placebo-controlled trial on TCM because they did not want to take the risk of being randomized to a placebo group. Subjects who believe the study herbal product may help them are likely to simply purchase the product from the market if it was commercially available. For incentive purposes we usually promise to provide the real medication free of change to participants who were randomized to receive the placebo at the end of the trial although this arrangement would not satisfy all.

In general, recruitment was difficult in clinical trials, and the refusal rate was high. A study reported (51) that 322 patients were screened and offered consent, but only 120 agreed to participate. Seventy-nine patients (39.2%) could not accept the placebo-controlled design, 45 patients (22.3%) had already been taking Chinese herbal preparation before starting chemotherapy, and the rest either refused without a reason or were not interested. According a report (49), 350 patients suitable for inclusion in the trial, 123 (35%) refusal to take part and 51 (15%) were excluded because they were unable to provide consent or relative assent.

Successful recruitment of participants is the key step to success in achieving the study aims. This paper described our reasoning, experiences, and the strategies we adopted to maximize recruitment during TCM clinical studies. Our experiences tell us that recruiting through the health services such as hospitals, clinics, general practitioners and nongovernmental organisations, is likely to be less successful than open advertising, as doctors in hospitals or clinics often have other priorities. Patient support groups may also be helpful. It is important that someone is assigned to

telephone duty to deal with initial contact to avoid loss of potential volunteers. TCM clinical trials require a wide range of skills to successfully recruit subjects, in particular organization and oral communication skills. It is important to think ahead and to pre-empt volunteer queries. Frequent contact, including briefing and debriefing meetings is essential for maintaining volunteer motivation to stay in the study. The newspaper articles could attract attentions of potential participants within a short period and was faster in efficiency and less expensive. However since the article or interview was only placed a few days in a week, it is not possible to estimate how many people were exposed to this, so overall response rates cannot be determined.

Drug and visit compliance

Medication compliance is a complex problem in health delivery (52), which can lead to unexpected or adverse outcomes (53). All age groups have been found to comply with only about 50% of drug and medical regimens (54).

Poor compliance can lead to iatrogenic illness and lack of medical efficiency.

Patients may take haphazard medication, or vary the dose, frequency or timing of their medication because of ignorance about the prescribed medication and its purpose (55). While product size often dictates the size of capsule chosen, it is important to choose the smallest capsule size possible. The larger a capsule is, the more difficult it is to swallow and the more likely it is for compliance to become an issue.

Dosage form also can influence the drug compliance. Our previous clinical trials on children with atopic dermatitis demonstrated that the compliance of syrup was much better than that of capsule. It is suggested that when we design a clinical trial for children we should consider the dosage form for better drug compliance.

Compliance may be affected by a specific outcome measure in trials of a drug for

example in osteoporosis clinical trial.

The methods we used for measuring compliance in our previous TCM clinical trials include checking the daily records issued at each visit and counting the residual study medication or return bottles at each visit.

In our osteoporosis clinical trial, TCM group compliance was less than placebo.

Table 7-16 Drug compliance

Group	Visit 2	Visit 3	Visit 4	Visit 5	Total
TCM	89.9%	77.3%	74.9%	75.8%	73.7%
Placebo	90 6%	86.1%	86.3%	84.1%	81.2%
P value	0.495	0.061	0.080	0.105	0.093

The total drug compliance in TCM group was 73.7%, lower than in Placebo group (81.2%, p=0.093), which may influence TCM therapeutic effects (**Table 7-16**; **Figure 7-19**).

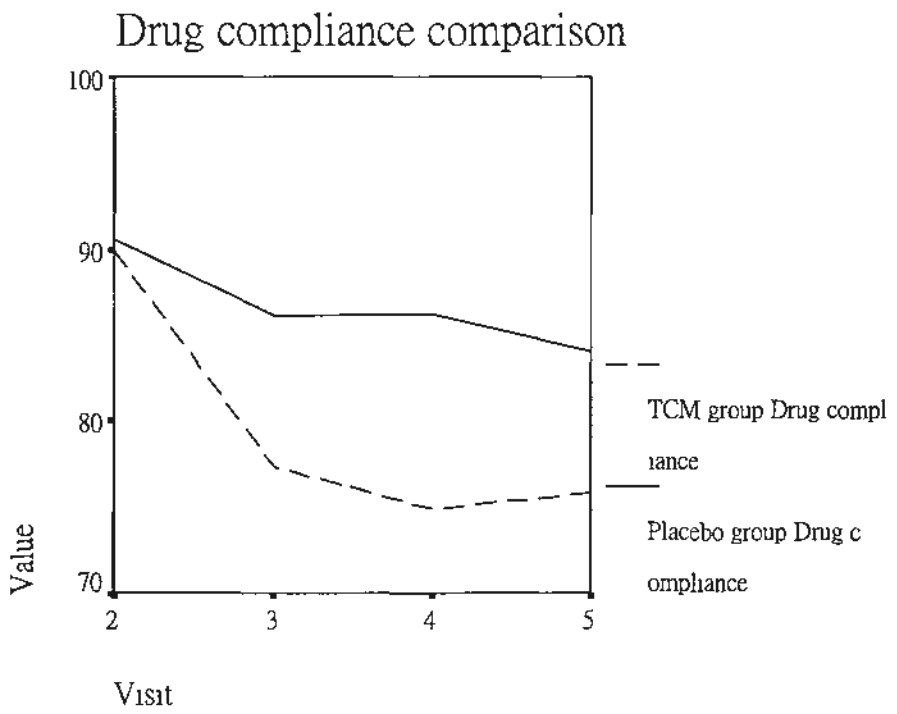


Figure 7-20 Drug compliance comparison between the two groups

Poor compliance with prescribed drugs can also jeopardise the outcome of clinical trials by reducing their power. Poor compliance is the most common cause of nonresponse to medication.

We discuss two possibly coexisting scenarios: (1) the participant takes an incorrect quantity of the study medication, and (2) the participant takes the correct quantity but in an incorrect manner (e.g., the wrong schedule of intake or the use of forbidden concomitant medication).

7.7 Implementation of clinical trial of Traditional Chinese Medicine

The importance of a well-designed protocol and a well-organized clinical trial cannot be overemphasized. The protocol ensures that patients are entered in a correct way to the clinical trial and that they are properly followed. An effective organization also assures that patients are not subject to unnecessary investigations and treatments.

The design of randomized clinical trial protocols have become fairly standardized due to the requirements of evidence-based medicine. **Table 7-17** shows the contents of our study protocols.

Table 7-17 Items of study protocol preparation

0	Title page	11	Statistical considerations
1	Background and introduction		Sample size calculation
	Natural history, prognostic factors		Statistical analysis procedures
	Rational, results of related studies	12	Interim analysis and early stopping rules
2	Objectives of the trial	13	Quality of life assessment
	Hypothesis to be tested	14	Cost evaluation assessment
	Endpoints	15	Data monitoring
3	Patient selection criteria (Inclusion/Exclusion		Composition
	Criteria)		Role
	Disease parameters	16	Quality assurance
	Informed consent	17	Ethical considerations
4	Trial design and Schema		Procedure for informed consent
	Trial design		Information to be provided to patients
	Schema	18	Investigator commitment statement
5	Therapeutic regimens, Toxicity, Dose Modification	19	Trial sponsorship/Financing
	Treatment regimens	20	Trial insurance
	Expected side effects	21	Publication policy
	Dose modification	22	Administrative signatures

6 Required clinical evaluation, Lab tests and Follow-up Prior to entry on study During the period of treatment During follow-up 7 Criteria of evaluation, Endpoints Definition of endpoints Handling of protocol violation During of follow-up 8 Patient registration and randomization procedure Administrative information Randomization checklist 9 Forms and procedures for collecting data 10 Reporting adverse events	23 List of participants with expected yearly accrual 24 References 25 Appendices Patient Medication Diary Card Functional Assessment of Cancer Therapy-General Scale Eastern Cooperative Oncology Group performance score Patient Information sheet Informed consent form Background Information of the study medication Case Report Form Drug storage/supply Adverse Drug Reaction

Conducting a TCM clinical trial is at least needed to go through the following 14 separate activities. These activities include:

(1) Protocol writing;

(2) Case Report Forms preparation;

(3) Investigator's brochure study;

(4) Pre-ethics committee submission activity;

(5) Study initiation;

(6) Subject recruitment;

(7) Informed consent;

(8) Randomization;

(9) Supply management and dispensing of the study medication;

(10) Clinic visits for distributing the medication;

(11) Clinic visits for follow-up care;

(12) Management, including office visits, related to adverse events;

(13) Data collection, management, analysis, and reporting;

(14) Pre-post study investigator meetings.

Recruitment is one of the most important steps in clinical trial, which is the key factor determining if a clinical trial can be completed on set schedule. Recruitment problems may occur at any point throughout a clinical trial. The necessary steps to counter this problem are dependent on the potential to expend groups of patients from the sources used, or to approach new sources (56). Successful recruitment to clinical trials requires other components unrelated to scientific and financial considerations. Success in reaching target recruitment depended largely on being able to directly contact patients through posters, newspaper advertisement, television interview, patient support groups and physicians referrals in hospitals. Suspicious of the placebo, and unwillingness to stop taking other herbal supplements made recruitment more difficult, more time-consuming, and costly. Considering the ethical principles and recruitment barriers, we recommend that clinical trials could be designed as Dose-dependent Trial using Low Dose as control to replace placebo.

7.8 Clinical trial data analysis

Statistical analysis report

When all the statistical analysis is completed, a complete statistical analysis report is usually needed. It is very useful in manuscript preparation. In the DBT Stage after all statistical analysis completed, we prepared a statistical analysis report for clinical trial report preparation. The following is the major components of the report.

Aim of study

To investigate the dose response relationship to assess in establishing an optimal dose for clinical use

Primary endpoint:

1) Daily recording of severity and frequency of hot flushes and sweats
2) Menopause Specific Quality of Life (MENQOL)
3) Greene Climacteric Scale

Sample size

Sixty (60) subjects divided into 3 groups in Stage II clinical trial.

Twenty allocated randomly to receive active treatment and 20 allocated to receive control treatment.

Parameters analyzed in the statistical report

1 General information

2 Self-reported data

- Hot flushes: frequency and severity

  Mild

  Moderate

  Severe

- Night sweats
- Severity of hot flushes: [(number of mild flushes × 1) + (number of moderate flushes × 2) + (number of severe flushes × 3)] ÷ total number of hot flushes on that day

3 Changes in MENQOL (four domains):

The MENQOL is a validated instrument that tests vasomotor, psychosocial, physical and sexual domains of quality of life. A minimum score of zero corresponds to no symptoms and

maximum score of six corresponds to extremely bothersome symptoms.

4 Greene Climacteric Scale data analysis

The total Greene Climacteric score for a given subject is the sum of all 21 scores. The mean score for each symptom is calculated by the sum of all individual scores divided by the number of subjects. The score of the clusters and the total score are given as the sum of the mean scores of the symptoms within that cluster and the sum of the mean scores of all symptoms, respectively.

5 Adverse events

Statistical methods used for data analysis

5 General information

Baseline characteristics were summarized by treatment group.

For continuous variables, means were compared using analysis of variance.

Categorical variables were compared using the χ^2 test.

6 Self-reported daily records (Hot flashes and night sweats)

Changes from baseline in the number and severity of hot flashes were assessed within treatment groups by using paired t-test. Comparisons between the three groups were conducted using One-way ANOVA.

The mean daily number and severity of hot flashes were compared between dosage groups for each month using an ANOVA.

Differences in categorical data between groups was explored using Mann-Whitney Test, Wilcoxon Signed Ranks Test or χ^2 test.

7 MENQOL and Greene Climacteric Scale

Changes in MENQOL and Greene Climacteric Scale scores from baseline

to month 3 and month 4 were analyzed with Paired t-test.

The difference between the three groups at each visit was analyzed by using One-way ANOVA.

8 Blood chemical data analysis

Student's t-test and ANOVA were used to compare the differences in results.

9 Adverse events

χ^2 test was used to compare the incidence between the treatment groups.

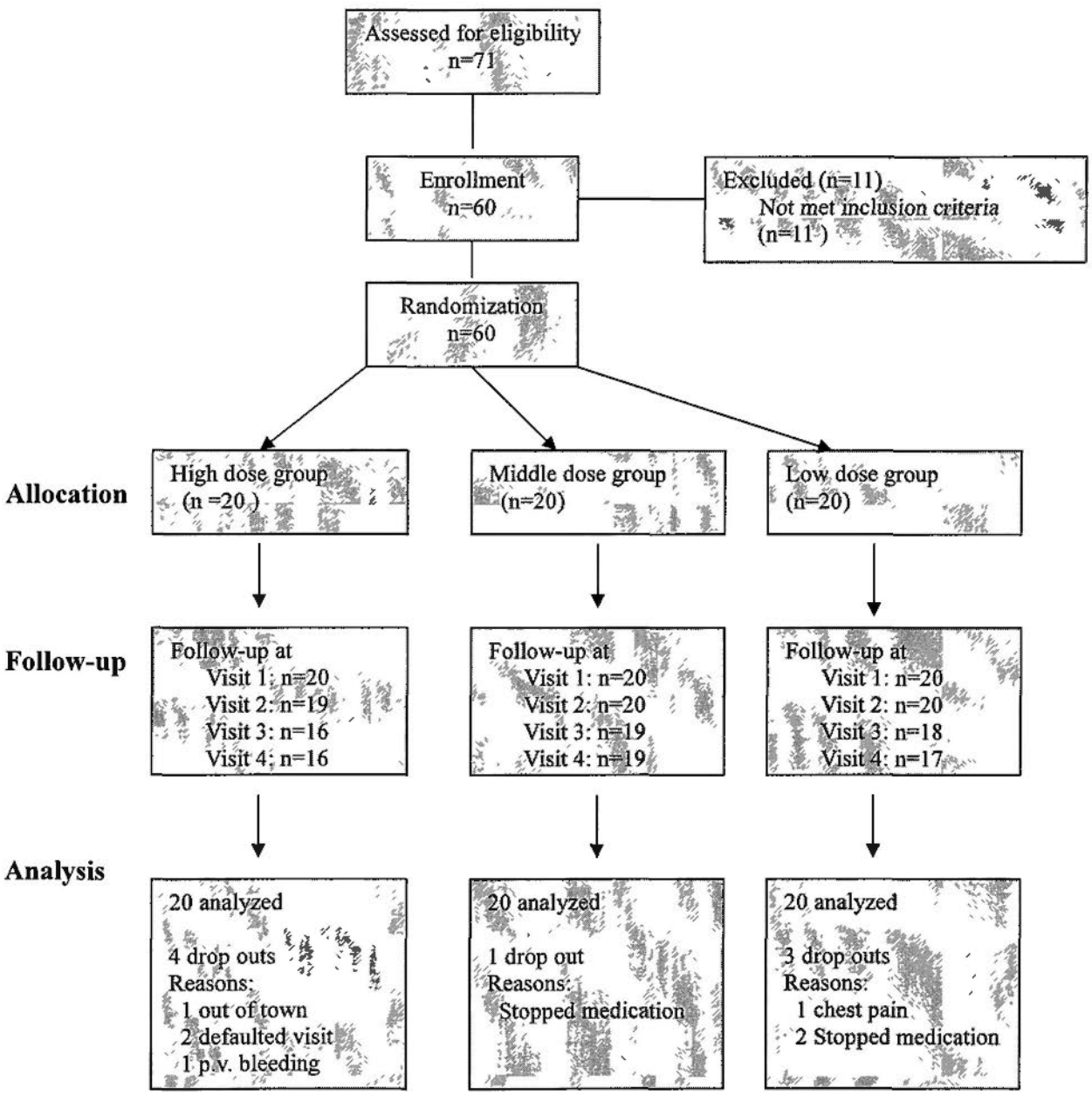


Figure 7-21 Stage II Flowchart

Table 7-18 Changes in Hot flush score (Greene Climacteric Scale, GCS)

Group	Screening	Visit 1 (baseline)	Visit 2	Visit 3	Visit 4 (follow-up)
High dose	3.10±0.64	3.10±0.55	2.68±0.67	2.47±0.62	2.44±0.62*
Middle dose	3.35±0.67	3.25±0.64	2.95±0.69	2.79±0.79	2.75±0.85
Low dose	2.95±0.69	3.10±0.55	2.72±0.75	2.76±0.66	3.00±0.79

**p=0.029 compared with low dose

As shown in **Table 7-18**, hot flush score of high dose group was lower than Low dose group after 3-month treatment (p=0.029) and the dose-response relationship was observed.

Table 7-19 Percent change in hot flush (GCS) from Baseline

Group	month 2	month 3	month 4
High dose	-11.8	-16.7	-17.6
P value#	0.005	0.003	0.004
Middle dose	-8.8	-13.6	-15.6
p value#	0.010	0.008	0.004
Low dose	-13.9	-12.8	-2.5
p value#	0.007	0.004	0.494

Refers to the comparison of each visit to baseline within group

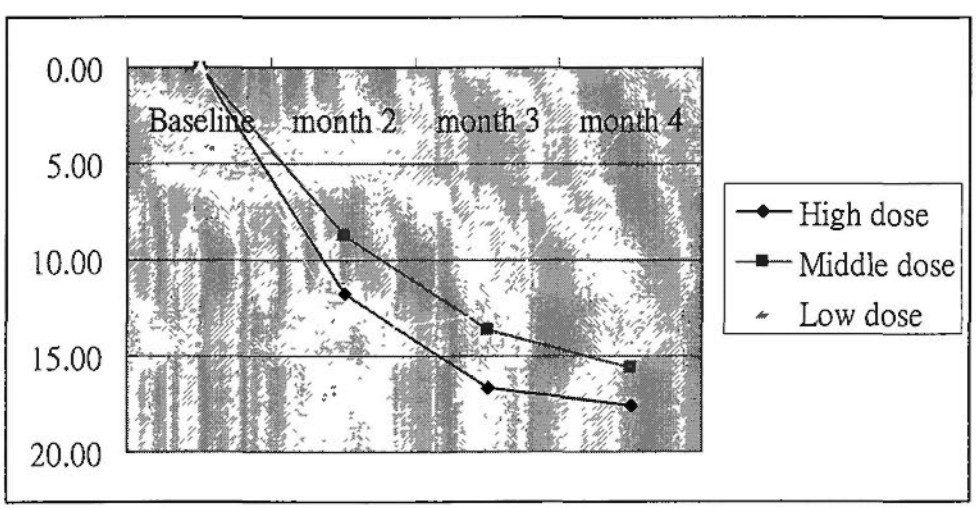


Figure 7-22 Percent change in hot flush (GCS) from Baseline

As shown in **Table 7-19** and **Figure 7-21**, there was a rebound in hot flush after stopping the treatment.

Table 7-20 Percent change in hot flush score (MENQOL) from baseline

Group	month 2	month 3	month 4
High dose	-32.1	-33.7	-32.0
P value#	0.000	0.000	0.001
Middle dose	-8.6	-18.3	-25.4
p value#	0.000	0.012	0.023
Low dose	-15.2	-16.2	-19.0
p value#	0.001	0.001	0.022

Refers to the comparison of each visit to baseline within group

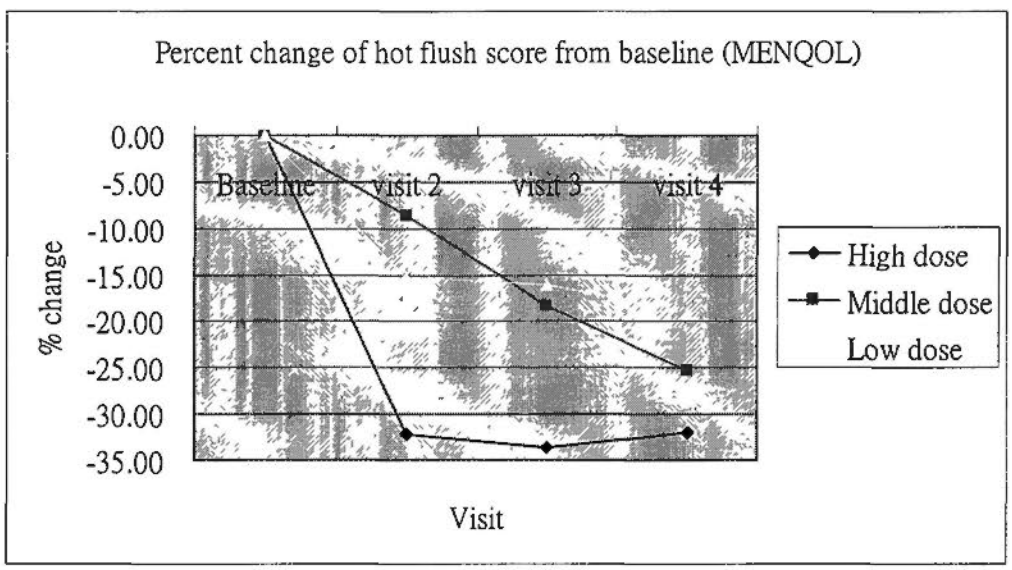


Figure 7-23 Percent change of hot flush score (MENQOL) from baseline

Table 7-21 Percent change in night sweat score (MENQOL) from baseline

Group	month 2	month 3	month 4
High dose	-29.8	-33.3	-38.6
P value#	0.003	0.001	0.001
Middle dose	-14.7	-17.7	-21.2
p value#	0.066	0.032	0.014
Low dose	-6.7	-7.6	1.9
p value#	0.509	0.391	0.808

Refers to the comparison of each visit to baseline within group

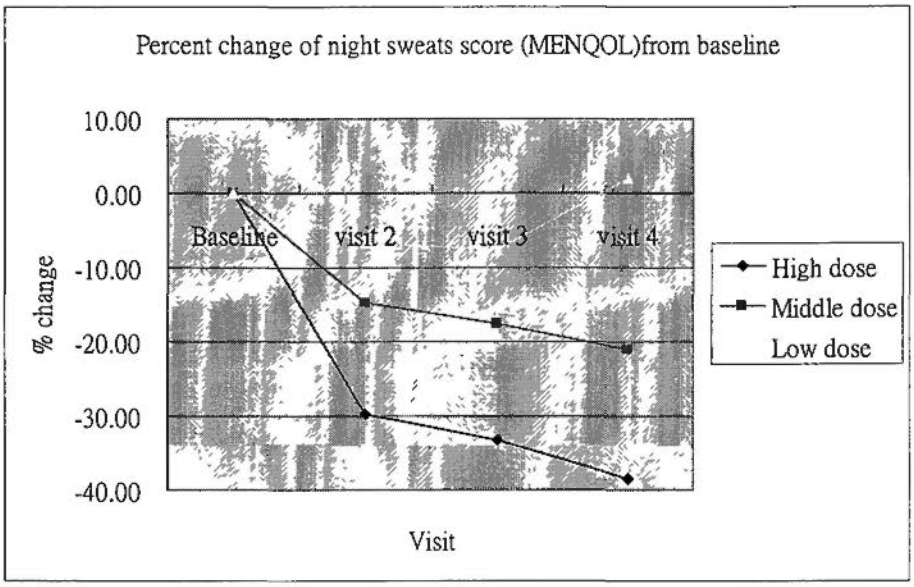


Figure 7-24 Percent change of night sweat score (MENQOL) from baseline

Table 7-22 Percent change in vasomotor cluster from Baseline

Group	month 2	month 3	month 4
High dose	-14.4	-18.2	-16.7
P value#	0.000	0.002	0.006
Middle dose	-7.9	-16.5	-18.6
p value#	0.029	0.002	0.001
Low dose	-11.8	-10.0	-3.4
p value#	0.014	0.024	0.408

Refers to the comparison of each visit to baseline within group

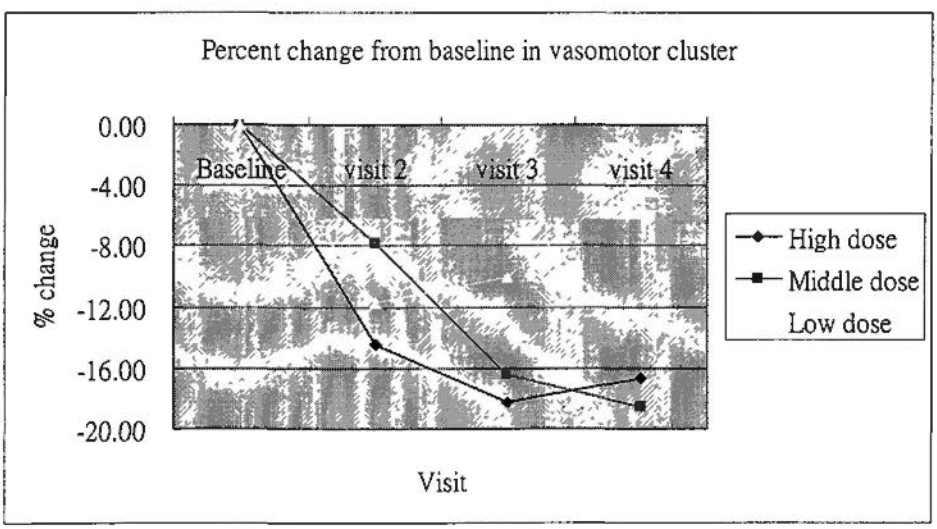


Figure 7-25 Percent change in vasomotor cluster from Baseline

Adverse Events

Table 7-23 Adverse Events during study period

Adverse Event	Group		
	High dose	Middle dose	Low dose
Sore throat	1	0	0
Post-menopausal bleeding	1	0	0
Hypotension	0	1	0
Tooth-ache	0	1	0
Chest pain	0	0	1
Swollen gum	0	0	1
Insomnia	0	0	1
Total	2	2	3

There was no difference in incidence of adverse events among the three dose group during study period (**Table 7-23**).

Conclusion

In the Multiple-Dose Escalation Study, the main purpose of the study was to look for an optimal dose for the treatment of menopausal symptoms.

Three groups were used: one group was treated with the normal dose which was the same as stage 1 trial; the second group was treated with a dose 100% higher than the normal dose and the third group with a dose 50% lower than the normal dose.

Low dose group: DBT 1.5g daily for 3 months

Middle dose group: DBT 3g daily for 3 months

High dose group: DBT 6g daily for 3 months

Dosage used in Stage I trial was only one-forth of traditional dosage (3g daily), which was demonstrated effective in control of mild hot flushes. Present study has demonstrated that high dose (6g daily) is more effective in control of vasomotor symptoms and improvement of quality of life.

We use another example "A randomized, double-blind, placebo-controlled study of the effect of Menoease Pills on menopausal symptoms and quality of life in Chinese women" to elucidate the statistical analysis report:

Statistical Analytical Preliminary Report

Title: 延采白凤丸改善更年期综合症的临床研究

(A randomized, double-blind, placebo-controlled study of the effect of Menoease Pills on menopausal symptoms and quality of life in Chinese women)

Parameters analyzed in the preliminary report

1 Hot Flushes per month at baseline and during study treatment

2 Changes in MENQOL (four domains)

- Vasomotor symptoms analysis

  Hot Flushes

  Night Sweats

  Sweating

- Psychosocial
- Physical
- Sexual

Software used in the analysis: SPSS 11.5 for Windows

Statistical methods used for data analysis

1 Hot flashes

Changes from baseline in the number and severity of hot flashes were assessed within treatment groups by using pared t-test. Comparisons to placebo were conducted using an ANCOVA wit baseline as a covariate.

The mean daily number and severity of hot flashes were compared among treatment groups for each month using an ANCOVA, with treatment as a factor in the model and baseline as the covariate.

Differences in categorical data between groups were explored by Mann-Whitney Test, Wilcoxon Signed Ranks Test or $\chi 2$ test.

A linear regression analysis was used to analyze the relationship between duration of menopause and the decrease in hot flashes during treatment.

2 MENQOL

Changes in MENQOL scores from baseline to month 2 and month 3 were analyzed with analysis of variance and analysis (ANOVA) of covariance (ANCOVA).

Results

1 Self-reported Data on Hot Flashes

Table 7-24 Number of Mild Hot Flushes each Month during Study Period

Month	1	2	3
Menoease pills	31.1	38.1	30.7
Placebo	33.9	33.0	35.3
P value	0.180	0.059	0.018

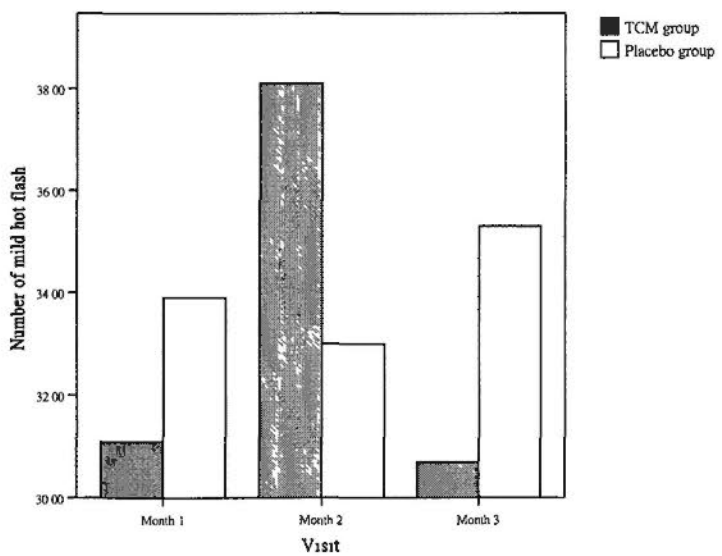


Figure 7-26 Number of Mild Hot Flushes Comparison

Table 7-25 Number of Moderate Hot Flushes each Month during Study Period

Month		1	2	3
	Menoease pills	51.0	31.4	16.6
	Placebo	49.2	47.5	40.8
	P value	0.472	0.000	0.000

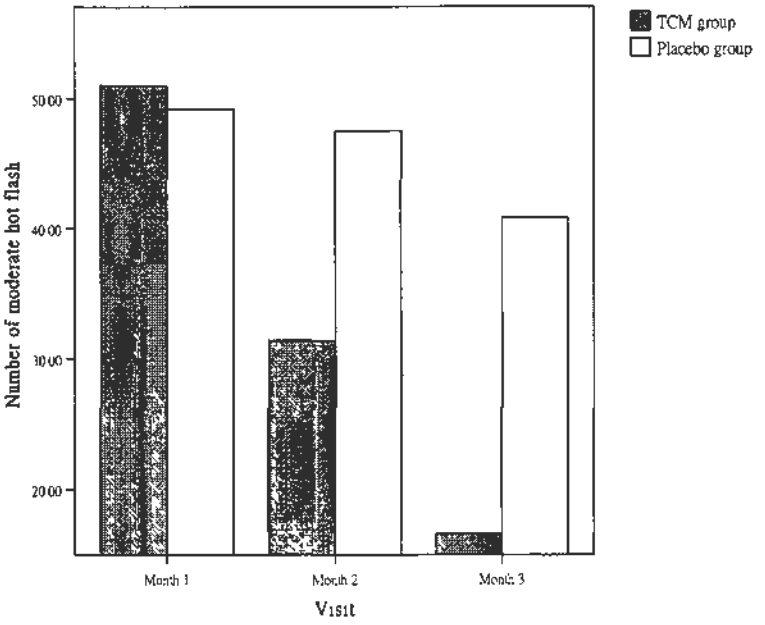


Figure 7-27 Number of Moderate Hot Flushes Comparison

Table 7-26 Number of Severe Hot Flushes each Month during Study Period

Month		1	2	3
	Menoease pills	4.7	1.3	0
	Placebo	3.0	2.3	2.7
	P value	0.103	0.364	--

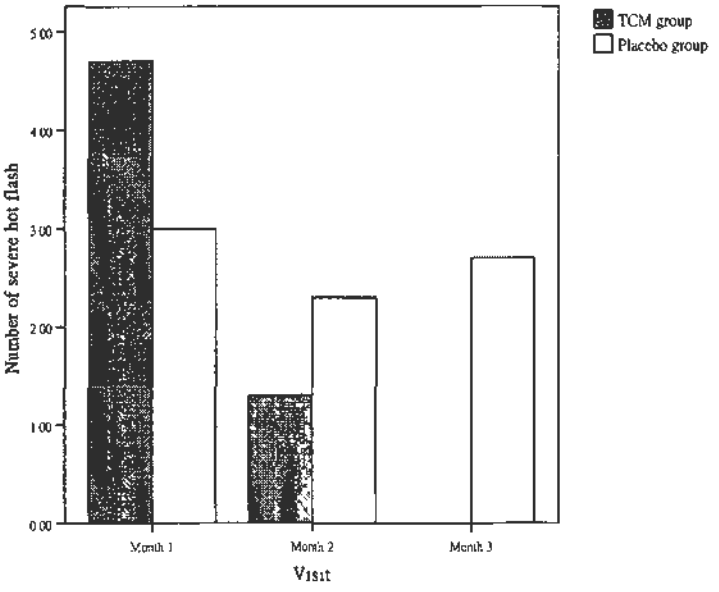


Figure 7-28 Number of Severe Hot Flushes Comparison

2 Score Changes of MENQOL

Table 7-27 Mean Score of MENQOL at each Visit **(N=60)**

	MENOEASE PILLS (% change from baseline)	**Placebo** (% change from baseline)	**p-value** MENOEASE PILLS vs. Placebo
2 Vasomotor domain			
Visit 1	3.53±0.89	3.36±0.57	**0.361**
Visit 2	2.66±0.77	3.17±0.59	**0.000**
Visit 3	1.82±0.79(-48%)	2.98±0.51(-11%)	**0.000**
3 Psychosocial domain			
Visit 1	2.15±0.74	2.20±0.68	**0.797**
Visit 2	1.81±0.62	2.00±0.58	**0.016**
Visit 3	1.24±0.65(-42%)	1.78±0.60(-19%)	**0.000**
4 Physical domain			
Visit 1	2.70±0.40	2.79±0.41	**0.420**
Visit 2	2.35±0.35	2.54±0.34	**0.003**
Visit 3	1.78±0.53(-34%)	2.38±0.31(-15%)	**0.000**
5 Sexual domain			
Visit 1	2.71±0.61	2.76±0.58	**0.783**
Visit 2	2.57±0.54	2.64±0.47	**0.523**
Visit 3	2.16±0.38(-20%)	2.56±0.43(-7%)	**0.000**

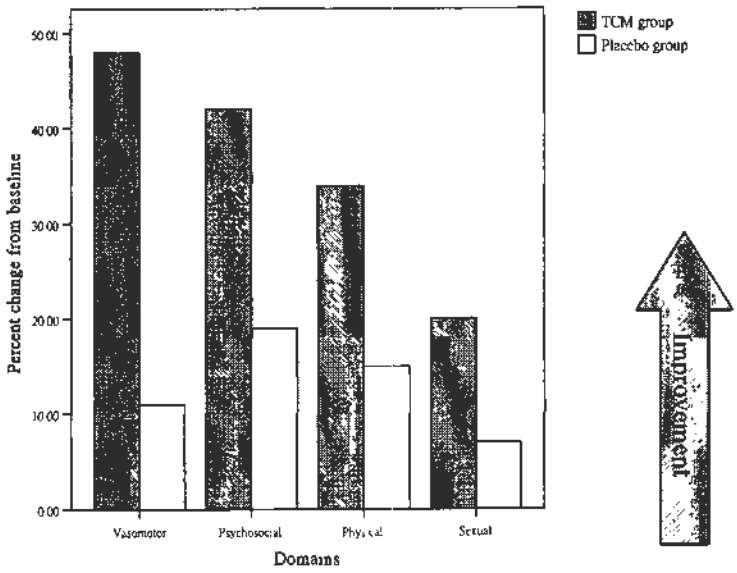


Figure 7-29 Percent change from baseline in each domain

Table 7-28 Vasomotor Domain Score Analysis (N=60)

	MENOEASE PILLS (n=30) (%)	Placebo (n=30) (%)	p-value (MENOEASE PILLS vs. Placebo)
6 Hot Flushes			
Visit 1	3.60±0.89	3.57±0.57	0.864
Visit 2	2.87±0.82	3.37±0.61	0.000
Visit 3	2.17±0.79((-40%)	3.03±0.49(-15%)	0.000
7 Night Sweats			
Visit 1	3.40±0.89	3.20±0.76	0.355
Visit 2	2.47±0.82	3.00±0.74	0.000
Visit 3	1.60±0.89(-53%)	2.90±0.61(-9%)	0.000
8 Sweating			
Visit 1	3.60±1.00	3.30±0.60	0.166
Visit 2	2.63±0.85	3.13±0.68	0.000
Visit 3	1.70±0.88(-53%)	3.00±0.64(-9%)	0.000

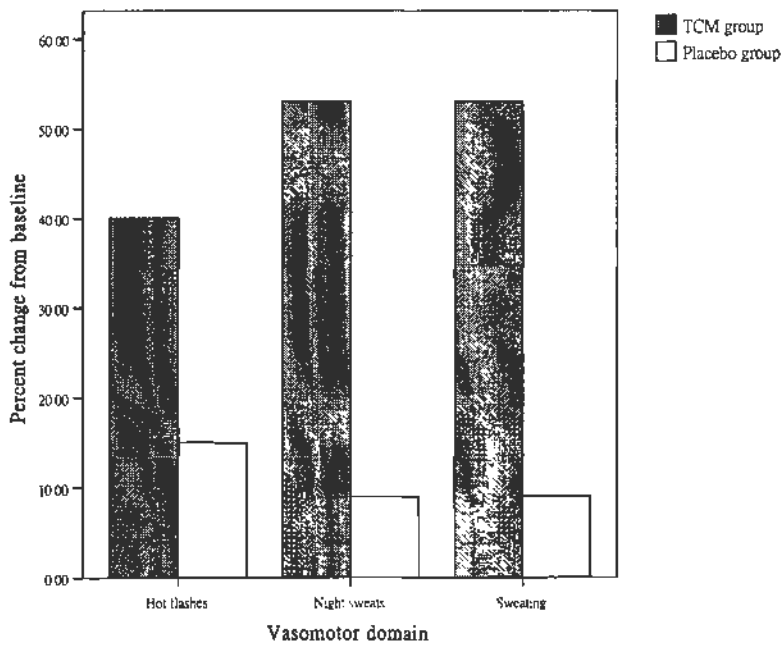


Figure 7-29 Percent change from baseline in vasomotor domain

Conclusions

The study results indicated that the study medication Menoease Pills has effect of

improvement in menopausal symptoms and quality of life of menopausal women.

Traditional Chinese medicine (TCM) has over 3000 years of history; however its evidence of efficacy is not well-documented. Strong evidence of efficacy should come from well-designed clinical trials which should be prospective experiments for assessing the results of medical interventions. The basic principle of a well-designed clinical trial of TCM should follow the same guidelines as those for Western medicine. A well-designed clinical trial must provide clear definitions or descriptions of research questions, participants and sample size, interventions, outcome measurements, randomization procedures, blinding, and statistical analysis. Traditional Chinese medicine is the practice of using combinations of herbs to restore the balance of a patient through a holistic approach. Unlike the Western medicine that is treating disease by pure chemical drug through targeting molecular or gene level, Chinese herbal medicines usually have their effectives through multi-targets. The philosophical frameworks are thus fundamentally different. In order to prove the efficacy of TCM, investigators should consider the principles accepted by TCM and Western medicine. The effects of TCM are usually mild and take a relatively long time to manifest clinically (57). This means that sample size of TCM clinical trial must be larger and the treatment duration should be longer that clinical trials of Western medicine. Most TCM clinical trials are conducted at our center, the study period is usually as long as 12 months.

The study project for the clinical development of DBT involved more than 140 patients and was designed to demonstrate the effect and optimum dose of DBT on menopausal symptoms in postmenopausal patients. This project was structured in two

studies: Stage I randomized placebo-controlled efficacy study, and stage II, dose-finding study. The clinical trials were set up with the highest quality standard to allow a thorough evaluation of the clinical benefits of DBT for postmenopausal symptoms.

Our experiences demonstrate that it is feasible to perform a well-designed TCM clinical trial although there are challenges to face, for example placebo preparation, outcome measurement and batch-to-batch consistency of TCM medication.

7.9 Clinical trial report writing

The quality of a clinical trial is depended on three aspects, they are trial design, implementation, and reporting. A well designed and implemented clinical trial may probably be considered as a low quality trial because the report writing is poor or incomplete, so that the authenticity of clinical trial results and clinical application are definitely affected.

In the mid-1990s last century, an international team composed by clinical epidemiologists, clinical professionals, statisticians and journal editors, was formed, and they spent nearly two years to prepare a norm to standardize the report of randomized controlled clinical trial. This standard is known as the "CONSORT (Consolidated Standards of Reporting Trials)" Statement [58]. Afterward, it was accepted by the international well-known clinical medicine journals.

Randomized controlled trial (RCT) is generally regarded as the "gold standard" design to assess the effectiveness of medical interventions. Well-designed and properly conducted RCT provides high-quality "raw materials" for conducting systematic reviews, health technology assessment and decision analysis reports.

Recently, generally accepted CONSORT was issued by the International Committee

of Medical Journal Editors (ICMJE) published in 2001, with the 22 universal clinical trial items)[59]. In view of the characteristics of herbal medicine itself, the direct use of CONSORT statement to standardize its clinical trials report existed defects. To this end, Gagnier and other experts revised and expanded the 22-item CONSORT to form " Reporting Randomized, Controlled Trials of Herbal Interventions: An Elaborated CONSORT Statement "[60]. It is used to standardize the report of herbal medicine clinical trial with a view of improving the quality of reporting herbal medicine RCT.

Because Chinese medicine is including a large number of herbs, so clinical trials of traditional Chinese medicine can be reported in accordance with the International Herbal CONSORT statement.

At present, there is no uniform reporting standards for TCM clinical trials. All professional journals of Chinese medicine have long formed their own style. There are slightly different requirements for submission in format and review. Some studies showed that overall quality of traditional Chinese medicine clinical trial report is still lower. It is necessary to make a specific standards to guide clinical trial report of traditional Chinese medicine〔61, 62〕.

Randomized controlled trials (RCTs) are widely accepted as the "gold standard" for providing evidence on the effectiveness of interventions. (1,2). To comprehend the results of RCTs, readers must understand the study design, conduct, analysis, and interpretation, which require total transparency on the part of the authors. In the mid-1990s, 2 independent initiatives to improve the quality of reporting of RCTs led to the publication of the Consolidated Standards of Reporting Trials (CONSORT) statement (58).

Chapter 8

Research Requirements Parallel to the Clinical Trials of Chinese Medicine

To conduct a well-designed clinical trial, some essential factors should be considered, such as placebo preparation and its verification, placebo effects during clinical trials, placebo effect and real drug effect, chemical markers and standard reference herbs for quality control and the quality assurance of herbal sources (GAP), and the control of manufacturing process (GMP). All these factors can affect the quality, safety and efficacy of herbal medication.

8.1 Chemical markers and standard materials

Drugs as a special commodity for disease prevention and treatment, must meet the basic requirements of "safety, efficacy, controllability and stability". In order to truly realize the modernization and internationalization of Chinese medicine, Chinese herbs, extract, granules, prepared Chinese medicine as the main forms of clinical medication, must also be consistent with the above-mentioned requirements. However, the unique characteristic of Chinese herbal medicine is reflected in the diversity and complexity of composition, instability of quality, ambiguity of mechanism, the difficulty in the control of production process, etc. To ensure the accuracy of clinical efficacy, it is necessary to ensure drug quality between batches in the stability and repeatability of production. Chemical markers and standard raw herbal materials are important basis to guarantee the quality of Chinese herbal medicine.

Chemical marker without therapeutic activity is adjusted to serve an analytical purpose. The chemical markers can be used to control batch-to-batch consistency of

the finished product. Chemical marker of Traditional Chinese medicine is the specific concept derived from the chemical marker of Western medicine. Chemical marker of Traditional Chinese medicine is an indispensable element in quality control.

Chemical markers can be divided into three levels:

1) International standard chemical markers: The chemical markers are prepared, calibrated and distributed by the International Chemical Marker Center of World Health Organization (WHO)

2) National standard chemical markers: The chemical markers are prepared, calibrated and distributed by National Institute for the Control of Pharmaceutical & Biological Products (NICPBP)

3) Operational standard chemical markers: Prepared by the relevant institutes according to the operational needs

Chemical markers of Traditional Chinese Medicine only have national standard chemical markers and operational standard chemical markers. Currently the supply of chemical markers of traditional Chinese medicine in China is very limited. The state has announced that there are more than 900 chemical markers of traditional Chinese medicine, however, NICPBP only provides less than 400 of them (9). It seriously affected the quality control and clinical efficacy study of traditional Chinese medicine.

Why TCM or natural medicine is clinically effective, there must be the material basis for its activity. The research of material basis of traditional Chinese medicine is fundamental in quality control and pharmacological efficacy. Especially the research of chemical markers provides material assurance in the quality of traditional Chinese medicine and its compound. However, due to long term of inadequate attention in basic research on chemical markers of traditional Chinese medicine and instability in

the quality of chemical markers of traditional Chinese medicine make the active ingredients of many Chinese herbal medicine are still unknown; it affect the consistency of quality and efficacy of TCM and increases the difficulty of clinical trial in accordance with GCP principles.

Standardization refers to measures taken to ensure that there is a consistent quantity of a defined marker compound within a herbal material, as herbal materials are known to be highly variable in their make up (10). Intrinsic factors (e.g. genetics) and extrinsic factors (e.g. growing, harvesting, storage and drying processes) may lead to variations in the chemical profiles of the herb (11, 12).

In order to achieve reproducible biological data in terms of safety and efficacy, it is important that the herbal material should be standardized to the active ingredients when they are known, or to specific markers when the actives are not yet known (13). Chemical fingerprints are commonly used to confirm the identity, authenticity and lot-lot consistency of a plant (14).

Herbal product studies cannot be considered scientifically valid if the product tested was not authenticated and characterized in order to ensure reproducibility in the manufacturing of the product in question. Many studies refer to the use of standardized material, but in reality they are referring to chemical standardization.

While chemical standardization is important, its utility is limited when the starting material is not well characterized botanically.

Each of the principal herbs should be standardized as to the content of the major active compounds (many of which might be unknown). The objective is to establish a chemical "fingerprint" that meets certain standards for each lot of a particular herb.

The actual formulation always will be based on a mixture of such standardized herbs.

Chemical markers which are used for drug quality control are divided into chemical drug markers and Chinese herbal drug markers. Chemical drug marker is often refined from raw material of the chemical drug that is easily available and inexpensive. Chinese herbal drug markers are hard to get and the price is expensive because of the raw herbal material constraints, and the complex process of isolation and identification. But it is very important and can not be avoided in traditional Chinese medicine research and quality control.

As a standard substance, there are certain criteria and specific requirements.

1) Purity: Chemical marker used for content determination the purity should be up to 97% or more;

2) Stability: Thermal and light stability. Such as marker Tanshinone Ⅱ A solution, at room temperature the content is continuously reduced with the increase in the number of placement days;

3) Uniformity: In the preparation of herbal markers of TCM, repeated crystallization often happens, which tends to uneven of the solution. So, it is necessary to mix the marker evenly before use (15).

The overall quality of a herbal medicine may be affected by many factors, including seasonal changes, harvesting time, cultivation sites, post-harvesting processing, adulterants or substitutes of raw materials, and procedures in extraction and preparation. From harvesting to manufacturing, chemical markers play a crucial role in evaluating the quality of herbal medicines. However, at present, some herbs do not have markers for quality control. According to the Chinese Pharmacopoeia (2005 edition), only 281 out of 551 herbs have one or two chemical markers for quality

control.

The study of chemical markers is applicable to many research areas, including authentication of genuine species, search for new resources or substitutes of raw materials, optimization of extraction and purification methods, structure elucidation and purity determination. Systematic investigations using chemical markers may lead to discoveries and development of new drugs.

In DBT project, *Radix Astragali* is graded according to its diameter, length and physical appearance. *Isoflavonoids* and *saponins* were recognised as the major bioactive components attributed to the therapeutic effects of *Radix Astragali*. These two types of components were used to evaluate the quality of *Radix Astragali*.

Determination of active ingredient is an important criterion for quality control. However, some contents are not really active ingredients, but landmark composition, representative ingredients or principal components; some are one or two ingredients of a variety of active ingredients, but not all active ingredients. It is insufficient to control the quality of herbs only based on the few markers. Some markers are only for measurement of the active ingredient, without the references for toxic and hazardous components testing. More importantly, a large number of unknown elements are completely out of control. Many unknown ingredients that may be active ingredients or toxic substances, are closely related to the drug's safety and effectiveness, but can not be effectively controlled.

Uniformed, normed, and stabilized quality standard is very important. But for many Chinese herbal medicines, the quality standards are not unified, non-standardized, and precarious. For example, the same drug in different dosage forms; or the same drug

and the same dosage form from different manufacturers; even in the same drug, the same dosage form, and the same manufacturer in different batches, all of these make the quality variable, the quality standards confusable.

8.2 Dosage form and dose of Traditional Chinese Medicine

Herbal extracts are usually made either in liquid, powdered or viscous forms from the crude mixtures of plant parts. The chemical compounds can then be extracted from plant material using water or organic solvents such as alcohol (ethanol). As a result, the extract contains only the soluble fractions and the non-soluble (fibrous) residues are discarded. Volatile oils are extracted by steam distillation or less often by solvent extraction. The herb to extract ratio (HER) is typically 5:1 for normal extracts.

Mixtures are products with medicinal properties which contain 2 or more plants or herbs that can act individually, additively or even synergistically to restore or maintain health. In Chinese, Indian and African Traditional medicines, medicinal plants are typically used in mixtures.

The dosage form of TCM usually includes decoctions, dried decoctions (granulas), Powders (capsule), Pills, tablets, syrup, and injection. Capsule, tablet and syrup are the commonly used dosage forms in our clinical trial centre.

Decoctions are generally inconvenient and unpleasant herbal preparations are used only as the situation might require. Decoctions were a primary method of therapy described in the ancient text *Shanghan Lun* (伤寒论); many of the conditions described in that text needed treatment, and there were variations of formulas that corresponded to each manifestation of the disease. Although duration of therapy was rarely indicated, it was evident that the decoctions were intended to be used for a day or a

few days, and that the patient was expected to be in a different status within a few days.

In the safety and efficacy evaluation of herbal medicines, an important consideration is the dosage formulation, which can affect the bioavailability of the active chemical constituents.

For oral administration, the liquid (syrup), capsules, tablets, or granular/powder are often considered. Each of these dosage forms has its unique characteristics, advantages and disadvantages.

Liquid and powdered dried extract dosage forms facilitate absorption, but the production of a placebo with the same taste and smell is very difficult. Capsules and tablets are the most popular oral dosage forms.

In the case of tablets, the presence of binders and excipients, plus the compacting of the mixture can lead to disintegration and dissolution problems, which in turn lead to questions of bioavailability.

Dosage of TCM is difficult to determine; this is because of the limitations in the knowledge of Chinese herbs. We often do not know the identity of the main active constituents or their quantities, nor how those quantities might vary among samples of the raw herbs, or how they might be affected by the way the herbs are prepared. Many people would think of herbs in terms of the pharmacological effects of the main active ingredients, in which case a certain dosage range will yield those effects, but lower doses may fail to give the desired results; this is a modern scientific viewpoint.

The typical dosage range for herbs in decoctions is relayed in the *Materia Medica* guides. For the majority of commonly used herbs, the range is 6-15 grams for a one-day dose, with an average of about 10 grams/day. Some herbs are used at considerably lower or higher dosages routinely, but these are the exceptions that must

be learned; some herbs are used in much higher dosage for certain applications. Decoctions are commonly made up of 8-16 ingredients, with an average of about 12 ingredients. Thus, a decoction of about 120 grams of crude herbs is usual (e.g., 12 ingredients x 10 grams/ingredient).

Why such large doses of herbs are needed. It is because all the herb materials in the formula are mild in nature. Only a relatively small proportion of the substances present might be deemed primary active ingredients of unique medicinal nature.

Decoction of herbs is a relatively easy but inefficient form of getting out active ingredients. When the dregs are thrown away, they are saturated in the decoction that is not consumed; as the water boils, aromatic ingredients dissipate into the air; other ingredients may be damaged during the prolonged heating (for example, substances bind together and become inactive because they are then not absorbed; other substances may oxidize and lose activity).

The inefficiency of decoction is made worse when careful attention is not paid to the different cooking times that might be appropriate.

Dried decoctions (granules) were developed in Japan in the 1950s as an alternative to decoctions and have become a major method of providing herbs in Japan, Taiwan, the U.S., and Europe.

The dried decoctions are produced by making large batches of either single herbs or traditional herb formulas as decoctions (in large tanks), and then draining the liquid from the dregs. The liquid is then evaporated (using heat and vacuum) to form a syrup. The syrup is then put into a spray-drier along with a powder carrier (usually starch or the dried, powdered, herb dregs), and the remaining water is evaporated, leaving a dry powder. There can be variations in this processing method, but it basically involves making a decoction and then drying it (freeze-drying is not used).

The addition of a carrier is very important because dried extracted herb materials will turn into a gummy solid or even a hard mass when exposed to even a small amount of moisture. Starch or other material prevents this from happening. The amount of this carrier that is needed depends on the herb used, but typically ranges from 25-50%, so this becomes a large portion of the product.

The amount of extractable materials also varies considerably from one herb to another and from one formula to another. The finished product, on average, is about a 4.5:1 concentration of the ingredients in the crude herbs. Put another way, it takes about 450 grams (about one pound) of raw materials to yield about 100 grams of finished product (a typical amount dispensed at one time). Some products may have a lower concentration, depending on the amount of carrier used and the herb or formula extracted. The manufacturers have been very reluctant to provide information on this important matter.

Powdering of crude herbs to make medicines has a long history, having been mentioned in the *Huangdi Neijing* (黃帝內經) and even the pre-*Neijing* scroll "*Wushier Bing Fang*" (五十二病方 about 168 B.C.). The use of powdered herbs reached two peaks in Chinese medical history, the first marked by the publication of the *Hejiju Fang* (和剤局方 1080 A.D.) which mainly dealt with premade formulas in powder and pill form, and the other being the current era in which powdering machinery and the desire for convenient forms of herbs combined with ecological concerns to focus attention on this method.

Renewal of dosage forms of TCM industrial products from the old to the modernized, from conventional pill to tablets, is a trend in current modernization of TCM industries. But it doesn't assure the renewed dosage forms with modified extraction

process be successful without any analytical evidence.

To meet the growing demand for consistency in herbal material handling and quality control, a new form of prepared medicinal herb, the granule of herbal extracts, has been developed and marketed in the major TCM hospitals in Hong Kong and mainland China. The prepared herbal granule is made by a modern technique of processing single medicinal herbs, in which herbs are boiled until a thick syrup emerges and then are dried by a combined spray drying and fluidized bed drying technique. Granules are considered to have the highest effectiveness among all the preparations, because they maintain the most active ingredients through such a process and retain potency for a long time in storage. Many TCM practitioners and consumers prefer to use such materials for combination formula preparations because granules are easier to keep and handle, and require lower amounts per volume than liquid extracts or raw materials. This new form of prepared medicinal herbs is being challenged by many conventional herbal drug manufacturers and researchers. One of the major critiques is that the use of such a modern preparation in traditional combination formulas omitted an important step, i.e., decoction of mixed raw herbal materials, where synergistic chemical interactions occur and they tend to enhance activity and reduce toxicity; this does not comply with TCM philosophy.

8.3 Placebos Used in Clinical Trials of Chinese Herbal Medicine

Randomized, double-blinded, placebo-controlled trials are considered the gold standard for all classes of medicine, and that all patients randomized to each arm receive uniform and consistent treatment. The placebo arm in a randomized clinical trial (RCT) allows for single or double blinding, which reduces bias, increases the internal rigor of a clinical trial (18). In many TCM therapies, it is simply not possible

to create a convincing placebo. To achieve the purpose of blinding, drug characteristics of real drug and placebo should be exactly identical in color, appearance, smell and taste. For chemical drug, placebo is only required to be odorless, tasteless and easy to manufacture. However, for TCM drug, placebo is very difficult to prepare. A complete blinding is nearly impossible because the unique characteristics of TCM medication are nearly impossible to mimic. Herbs can be processed to make extracts, concentrated powders, or granules, which can then be prepared as tablets or capsules to facilitate the development of a convincing placebo (19, 20), but whether the placebo can meet the requirements of blinding need to be evaluated.

In the past two decades, more and more clinical trials on Traditional Chinese Medications have been designed and implemented in compliance with the Randomized Controlled Trial (RCT) standards. Almost all placebo-controlled clinical trials are designed with double blinding to eliminate the potential influence of non-pharmaceutical effects, which include the natural course of the disease, expectation of the subjects and investigators, as well as subjective factors in treatment, diagnosis and clinical assessment. (21, 22, 23, 24, 25, 26).

It is important that a placebo should be designed to appear smell, and taste indistinguishably from the real preparation while pharmaceutical activity and toxicity were both absent. However, if a placebo is easily identified, it is doubtful whether a double blinded design could be implemented; the quality of the clinical trial will be affected. (27, 28)

Chinese Herbal Medicine preparations carry special macroscopic, sensory characteristics including appearance, weight, size of particles, color, smell and taste. The complexity makes a perfectly matching placebo nearly impossible. However, this is an issue that needs to be addressed seriously.

We electronically and manually searched *Wangfang* database (万方数据), which contained 827 Chinese journals in medicine and pharmacy dated from 1999 to 2005, and found 598 full-length articles which mentioned the use of placebos. Seventy seven placebo blinded clinical trials for Chinese medicine were extracted from the 598 articles. We found that nearly 50% of the 77 clinical trials did not mention the macroscopic and sensory characteristics, such as color, smell, taste, texture, shape, and size etc., of the herbal medication under test and placebo, and did not evaluate if they were comparable. The language of the articles was mainly Chinese but a few were written in English.

These Seventy-seven articles were reviewed according to the principle that placebo should be identical with the study medication in macroscopic and sensory characteristics and the results were summarized as follows:

(1) 75 articles (97.4%) did not pay attention to the comparison of the placebo with the study medication (29-62). Instead, only very limited information was supplied (63-103).

(2) Only 2 articles (2.6%) specified the comparability and validity of the macroscopic and sensory characteristics between the study preparations and the placeboes (104, 105).

Table 8-1 gives examples of some reviewed articles which reported on placebo controlled TCM trials.

Table 8-1 Some reviewed articles

	Reference	Study design, indication	Contrasting identity of placebo with the study drug	Evaluation of the placebo preparation
Group I (4 papers) No reference to placebo	38	RCT, congestive heart failure	No mentioned	Not done
	41	RCT, postmenopausal osteoporosis	No mentioned	Not done
	53	RCT, chronic prostatitis	No mentioned	Not done
	62	RCT, peptic ulcer	No mentioned	Not done
Group II (4 papers) Nature of placebos given but no evaluation	64	RCT, cholelithiasis	EBHM and placebo (both tablets look the same)	Not done
	67	RCT, memory function letdown	Tiao Xing Fang, Bu Shen Fang were herbal decoction in brown color Placebo was tablets made of white starch with identical in appearance and weight	Not done
	70	RCT, cognitive impairment in Schizophrenia	Prepared by caramel as adjuvant in same color with Jian Pi Bu Shen He Ji	Not done
	93	RCT, analgesic effect	Negative control: placebo similar to Xie Du injection in appearance	Not done
Group III (2 papers) Nature of placebo given with evaluation	104	Placebo preparation in TCM clinical trial	Subjects without doubt in appearance, color and taste during study period	10 formulation staff were invited to evaluate the placebo by scoring the visual appearance, color and taste
	105	RCT, chronic heart failure	Shen Mai Capsule and placebo are identical in label, container and capsule	Assessors were invited to judge according to physical property of either placebo or testing drug.

It might be worthwhile going into some details of the two papers which made special attempts to justify the placebos being used.

In the first paper, Wang Xue-feng (104) reported that proprietor Chinese medicine had its own special sensory specifications such as taste and smell, and artificially copying

the characteristics in the placebo was very difficult. Attempts to prepare the placebo for *Xiao Er Qing Fei Yin* (小儿清肺饮) was discussed in the paper. Unfortunately only color was matched. In the second paper, Wen Ze-huai (105) expressed that validation of a placebo was rare in mainland China. The report evaluated the placebo of *Shen Mai Capsule* (生脉胶囊) in label, appearances of the container and the capsule in comparison with the real drug. 22 volunteers were invited to independently assess the identity of the placebo and study drug. The results showed that the right and accuracy of judgment for the placebo and real drug was similar.

The review showed that nearly half of the clinical trials did not describe the sensory specifications, the identity and comparability of the study medication and the placebo. Only a few reports (2.59%) simply stated the comparability. Apparently, the investigators did not entirely recognize the importance of proper placebo. A well prepared placebo-controlled clinical trial should satisfy three fundamental requirements, viz.: (1) evaluating the validity of a placebo controlled trial; (2) assessing the placebo in macroscopic and sensory characteristics; and (3) describing the preparation methods of placebo. An optimal placebo should meet the requirements of RCT. This may be not a problem for chemical medicine. However, it is very difficult for Chinese herbal medicines because of their unique characteristics. Commonly used materials for producing CHM placebo include maize powder, food color, and taste additives (104).

A perfect placebo should be identical with the study drug in all sensory specification without the pharmaceutical activity of study drug. In Western medicine research, placebo used for clinical trial is usually made by starch, which can be identified without difficulty if needed. But it usually does not affect the quality of clinical trial. For TCM clinical trial, a perfect placebo is unnecessary, in fact, impossible. What we

need is a proper placebo, which resembles as far as possible, the study TCM in the appearance, weight, size, color, taste and smell.

So far there are very fewer papers in evaluation of the placebo used in CHM clinical trials. It is time that TCM researchers should reach at a consensus about the criteria that would be required for the proper evaluation of the placebo of Chinese medicine.

8.4 Placebo evaluation and verification

Randomized controlled trials (RCT) have now been recognized as the gold standard for interventional clinical trials (106). The results obtained from RCT are considered as the high-level scientific evidence (107). In the randomized controlled trials of Chinese medicine, placebo-controlled trials only accounted for 2.09% (108).

Blinding in clinical research is considered an important method in ensuring the quality of clinical trials, which could reduce ascertainment bias, information bias, and observer bias (109, 110, 111, 112). Lack of blinding amongst the study personnel administering interventions may lead to performance bias as well as biased choices regarding analytical strategies and methods used (113).

Since the establishment of evidence-based medicine, clinical trials have followed a standard methodology, which consists of randomization of trial clients into the treatment and non-treatment groups, usage of placebo for the non-treatment and keeping the working personnels as well as the people under trial blind to the randomized results. One essential requirement for the success of such trial is to supply a placebo, which resembles the real drug, to the non-treatment group, so that the blinding principle could be kept (114). Without the matching placebo, blinding cannot be perfect and the true value of the clinical trial would be devalued (113).

The provision of a matching placebo in chemical drug trials could be easy and straight

forward since the chemicals in the pharmaceutical under test could be perfectly matched with inert material, likewise the preparation itself, in terms of physical form, external criteria and other sensory perceptions, could also be perfectly matched. Using herbal extracts as objects of clinical trial, however, could be met with a lot of practical difficulties when a matching placebo is required. Herbal extracts are invariably provided with special colours, special smells and unique tastes. To match these sensory perceptions with a placebo would not be a straightforward task (114).

60.17% of literatures described the production of the placebo, but it was difficult to determine the similarity of the TCM preparation and placebo in all aspects (108).

In a RCT study, a matching placebo is required. The criteria of placebo include the following:

1) Physical Form
2) Chemical Analysis
3) Sensory Perception
4) Packaging, and
5) Labeling

We take D&G clinical trial designed as randomized, double-blind, placebo controlled trial as example to determine if the placebo capsules were indistinguishable from the real herbal drug D&G (*Danshen* (Salvia miltiorrhiza) and *Gegeng* (Radix puerariae)) capsules.

The clinical trial requires a uniform preparation with standard dosage and duration of treatment. The uniform preparation used in the study was a capsule, which filled with a standard weight of dry powder produced from the decoction that came out from the boiling of *Danshen* and *Gegeng* in the selected proportions. The color of the extract powder was brownish and the smell was a unique pleasant herbal smell. The taste was slightly bitter and a little sweet.

The production of the matching placebo followed the following procedures:

1) Physical Form—the capsule

A 500mg capsule was used. The quality of the capsule satisfied the requirements set by the Department of Health of Hong Kong (115). The 0# size capsule with blue cap and light blue capsule body was used for both placebo and real drug. The placebo capsule was filled with starch and caramel. The capsule was weighed 500±50mg and the water content was less than 10%. (**Figure 8-1**)

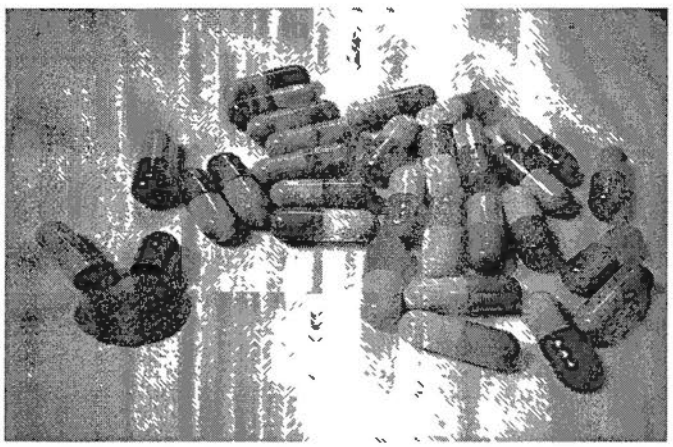


Figure 8-1 Placebo of herbal preparation

2) Sensory Perception of capsule and its contents

The capsules used for the herbal powder and placebos were exactly similar in appearance (**Figure 8-1**). Preparation of the placebo powder, different proportions of the components were tried to make the color, smell and taste closely resemble to the herbal preparation. A simple trial-and-error technique was used to assess the right proportion of the different ingredients. (**Figure 8-2**)

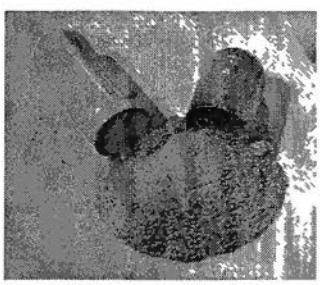
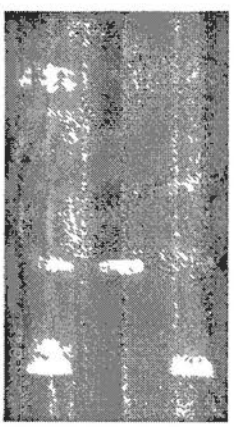


Figure 8-2 Contents of herbal capsule & placebo capsule

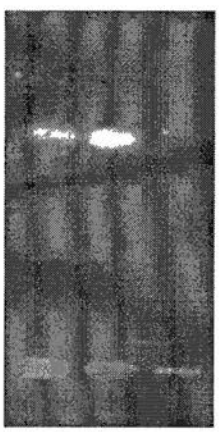
3) Chemical Analysis

In design of a placebo, it is essential that the placebo should be lack of pharmacological activity. It should be inert and suitable for use in clinical trial. In selecting an appropriate test system, considerations should be given to the sensitivity, reproducibility and general acceptance. Chemical analysis is to test if the placebo powder contains the active ingredients. The *Danshen-Gegeng* extract was put on a Thin Layer Chromatography (TLC) to analyze the active marker compounds under the condition of UV365 nm. The fluorescence image obtained through the TLC process using the extract was compared with the image obtained from placebo material. Reference image for *Danshen* (Tanshinone) and *Gegeng* (Puerarin) were also used to absolutely rule out the presence of the herbal extracts. (**Figure 8-7**)



1 2 3

1.GD capsule 2.Puerarin (reference)
3.Placebo



1 2 3

1.GD capsule 2.Tanshinone IIA (reference)
3.Placebo

Figure 8-3 TLC analysis for active marker compounds

The results showed that the placebo powder contained no any chemical markers that D&G powder contained (**Figure 8-3**).

4) Packaging

Both inner and outer packagings were carefully prepared in order to reach uniformity between the herb preparation and placebo.

a Inner package

White round plastic bottles of 130ml with special caps were used. The bottle was sealed with a heat-sealed paper top A standard desiccant pack containing round transparent desiccating pearls of diameter 1.5 mm was kept in each bottle. (**Figure 8-5**)

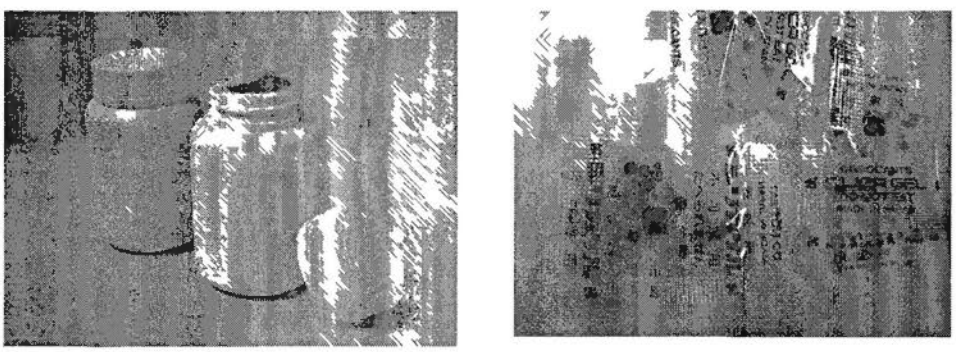


Figure 8-4 Inner package

b Outer package

Light brown rectangular cartons 50cm × 30cm × 25cm, sealed with brown adhesive taps were used. Both herbal and placebo were packed with identical means.

5) Labeling

In placebo controlled trials it is necessary to present all supplies in consistent packaging as well as labeling for maintaining blinding. The label for both herbal preparation and placebo contained the name of the research institution, project title, the patient's name, gender, patient's assigned number and part of his / her identity card number, the number of visits, dates, as well as the usage instructions. The label specifications of placebo were identical with herbal capsules', by means of printing styles that included

- Text of label content, font, size, color, format
- Icon of label: color, size, position of icon

- Material of label: texture, color, shape, size
- Position of label: sticking the labels on the same position

a. Packaging

After completion of essential procedures for the provision of herb preparation and placebo, a panel of fifteen adult numbers was selected to give a final check on the genuinity of the placebo and its blinding effects. The numbers, blinded to the contents of the packages were randomly given the samples of the packages. Each one was required to examine the box and bottle, and give an answer that whether they could identify the herbal preparation or placebo (116 //117). The panel numbers were fully aware of the purpose of the evaluation, their rights and obligations (118). (**Figure 9-9**)

Figure 8-5 Evaluation members doing separate random checking

Results of the evaluation were shown in **Table 8-2**. The scoring was made by means of visual and tactile according to the items listed in **Table 8-2**.

Table 8-2 Package and labeling evaluation

Evaluation Item		The independent evaluation score (Exactly identical = 3, very close to unanimous = 2, significant difference = 1, inconsistent = 0)															Average
(1) outer pack material	texture	3	3	3	3	3	3	3	-	3	3	3	3	3	3	3	3
	color	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	shape	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	size	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

(2) inter pack material	texture	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	color	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	shape	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	size	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
(3) desiccant pack	icon	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	color	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	shape	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	size	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
(4) label text	content	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	font	3	3	-	3	3	3	3	3	3	3	3	3	3	3	3	3
	size	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	color	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	format	1	2	3	3	3	3	3	3	3	3	3	3	3	3	3	2.8
(5) label icon	shape	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	color	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	size	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	icon position	3	3	3	3	3	-	3	3	3	3	3	3	-	3	3	3
(6) label material	texture	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	color	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	shape	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	size	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
(7) label adhesive position		3	2	3	3	3	3	2	3	3	3	1	3	3	2	2	2.7

The results showed that average scores were over 2, which indicated that herbal drug and placebo packages were identical in package and labeling.

b. Capsules

Another panel of adults, 5 men and 6 women, were invited to assess the capsules from randomly selected bottles, using visual and tactile scoring. Capsules were opened up to allow visual assessment of the powder and to detect the smell. No tasting was allowed because the taste was not adjusted with bitter agent that might have pharmaceutical effects.

Table 8-3 gives the results of the evaluation of the capsules.

Table 8-3 Consistency of capsule form

Evaluation Item		The independent evaluation score (Exactly the same = 3, very close to unanimous = 2, with significant difference = 1, inconsistent = 0)											Average
(1)	texture	3	3	3	3	3	3	3	3	3	3	3	3
surface	color	3	3	3	3	3	3	2	3	3	3	3	2.9
features	shape	3	3	3	3	3	3	3	3	3	3	3	3
	size	3	3	3	3	3	3	3	3	3	3	3	3
(2) fillers	particle	2	3	1	3	3	1	3	2	3	2	3	2.4
features	texture	3	-	2	3	-	3	3	3	2	2	-	2.6
	color	2	3	1	2	1	3	2	3	2	2	2	2.2

The results showed that the average scores were over 2, which means that herbal capsule and placebo capsule were identical. The evaluation results satisfied the requirements of placebo assessment criteria.

c. Overall results

Table 8-4 gives the overall results of evaluation.

Table 8-4 Overall testing results

Groups	Outcome of Judgment (panel numbers)			
	Correct	Incorrect	Unable to judge	Total
G&D Capsules	8	4	1	13
Placebo	4	4	2	10
Total	12	8	3	23

In this study, the overall results (Table 3) were analyzed by using the Chi-Square Test. The hypothesis was that "The members found herbal capsule or placebo identical". SPSS 14.0 was used for the data analysis.

The calculated χ^2 was 1.307, P= 0.520. The statistical outcome suggested that there was no different between the two groups. Based on this data, it appeared that the panel members were indeed blinded to the real nature of the capsules that they examined.

Today, blinding and randomization are considered the two important aspects of a good, well-controlled clinical trial (119). To satisfy the basic requirements of a double-blinded clinical trial and to reduce the bias, a well prepared placebo is critical.

Unfortunately, so far there were no recommendations on proper evaluation of the reliability of placebo for randomized double-blinded clinical trial of herbal medicine. This study was intended to establish an assessment method for the quality and validity of placebo specifically for herbal preparation. The assessment criteria included packaging, labeling, capsule specification, marker / active compound detection and sensory evaluations. The marker / active compound detection might be the most essential part among the assessing parameters to ensure the absence of interference. The placebo capsule must be identical to the study herbal capsules but without the marker / active compound and thus absent biological effects. Other evaluation criteria were also important to ensure real blinding and avoiding psychological biases. The overall results of sensory assessment demonstrated that the criteria used in this study could be endorsed as standard in the testing of herbal placebo.

Although the testing results revealed that the placebo under test was satisfactory in all aspects of assessment and could serve as reference for future clinical trials using placebo, nevertheless, each TCM medication has its own characteristics, as well as its different dosage form. So some of the evaluation items should be adjusted according to the nature of particular medication. With other forms of preparation, e.g. tablets and granules (or powder), modification in the evaluation of the simulating placebo should be considered accordingly. In the latter cases, even the taste of the herbal product would need to be matched. However, in our study we did not adjust the taste with bitter agent because some bitter agents may have pharmaceutical activities, which might interfere with the trial results.

As some Chinese herbal preparation such as *Danggui* carrying a special smell, it is difficult to prepare a placebo that the smell, colors are fully consistent with this kind of real drugs. Using 5.0% - 10.0% of the low concentration of the herbal drug as placebo is worthy of consideration.

In our usual practice, putting the cotton into the bottles of real drug for a period of time for sucking the flavor of the real medicine and then place the cotton into placebo bottles is a alternative solution for placebo preparation, as a result the taste of real drug and placebo is alike not only in appearance but also in the flavor.

8.5 Placebo effect

Placebo effect is prevalent in medical practice; it is particularly evident in traditional Chinese medicine research. The so-called placebo effect refers to the expectations of symptoms or illness alleviation or improvement when provided to patients with the placebo during the treatment period. The placebo effect was produced through the awareness, feeling, and taking the medication, as well as through the mental - physical interaction. Many studies showed that at least one-third of people responded to placebo, and the clinical symptoms were improved (120).

Placebo effect and the pharmacological effect are different, the former has no dose-response relationship, and different individuals may show the different effects. Placebo effect usually produces beneficial impact on the body, and there are certain effects on certain diseases by alleviating the symptoms, it is called positive placebo effect. The role of placebo for pain relief is most definite, for example, analgesic efficiency of up to 60%.

Beecher analyzed 1082 cases in clinical trials and found that an average of 35% of patients could benefit from the placebo (121).

In clinical trials, about 20% to 30% patients had very strong response to the placebo (122).

In the poor quality controlled clinical trials of Chinese medicine, selecting a positive reference drug as control often come to the conclusions that the control group and treatment group are of equivalence, it objectively reduces the risk of failure of the

clinical trial. Pharmaceutical enterprises from the perspective of their own economic interests are often willing to choose a positive control, and not much concern the quality control. Instead, choosing placebo as control is highly possible to draw ineffective conclusions if the quality control of the clinical trial is not strict. Thus in this sense, pharmaceutical companies and clinical research institutes will be more concern the quality control of clinical trial if the choice of placebo as a control.

Researchers have found that a medical encounter may produce its own placebo effects that can bring significant symptom improvement. The part of the encounter that plays the greatest role in the placebo effect appears to be nonspecific effects in the patient–physician relationship, including attention; compassionate care, and the modulation of expectations, anxiety, and self-awareness. In theory, the placebo effect of a medical encounter can be divided into the response to three main components:

1) the assessment and observation,
2) placebo treatment, and
3) patient-physician relationship.

The placebo effect involves five components of: patient, practitioner, patient–practitioner interaction, nature of the illness, and treatment and setting

The term placebo effect is taken to mean not only the narrow effect of an imitation intervention but also the broad amalgam of nonspecific effects present in any patient–practitioner relationship, including attention; communication of concern; intense monitoring; diagnostic procedures; labeling of complaint; and alterations produced in a patient's expectancy, anxiety, and relationship to the illness.

Evidence shows that patient expectations influence outcomes of both placebo and active treatment (123, 124, 125).

8.6 Distinguish of placebo effects and real medication effects

In TCM clinical trials, we found that the placebo effects could be more remarkable. We conducted a TCM clinical trial aimed to study the efficacy and safety of a herbal formula named SBL in patients with moderate to severe perennial allergic rhinitis. In the clinical trial, 126 allergic rhinitis patients were recruited in a double-blind randomized control trial. Half of the patients received SBL capsules and the others received placebo for 4 weeks. Symptoms scores, physician's evaluation, nose examination, quality of life, adverse effects, serum cytokines were evaluated before and after treatment. SBL was found to be effective in relieving some symptoms of perennial allergic rhinitis, improving the nose condition, and enhancing some domains of quality of life when compare to placebo, ($p<0.05$). In the 2 weeks follow up after treatment completion, SBL enjoyed a prolongation of symptom control ($p=0.05$). However, in the placebo group symptoms rebounded (**Figure 8-10**).

To lower the placebo effects, the design of the trial could include more assessment parameters in the first place. The optimized design is to have a third group of no treatment, parallel with the treatment and placebo groups. Or alternatively, the base line observation could be kept longer to get more thorough information about the clinical situation before any treatment started (126).

Extending the observational period after the drug treatment is over, to realize whether effects would be sustained, would be a useful compromise. In addition, allow the placebo group to shift over to open treatment, or simply extend the treatment for a reasonable period, could be a practical ways to attract more useful information.

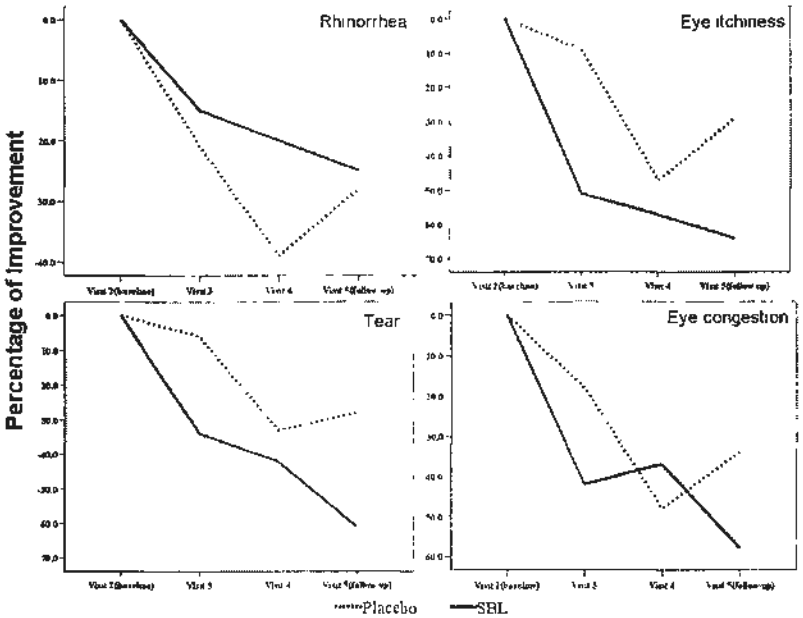


Figure 8-6 Improvement of Visual Analogue Scale (VAS)

As the figure 9-10 shown after the treatment was completed, each subject was followed up for 2 weeks and the visual analogue scale (VAS) was also evaluated at the end of follow-up period (visit 5). The results indicated that in the two weeks when no treatment was given, symptoms rebounded in the placebo group. However, in the SBL group all symptoms except nose blockage continued to show improvement due to the TCM residual effects.

Placebo-controlled trials aim to show that the experimental treatment is superior to the "nonspecific" elements solidified which is assessed through the use of a placebo arm. Placebo-controlled trials intend to prove if the experimental TCM drug has specific or pharmacologic effects. However, the effectiveness of TCM medication is usually nonspecific. Hence, in placebo-controlled clinical trial, the experimental TCM drug with nonspecific effects is compared with the "nonspecific treatment" and not with the theoretical "nothing" of the placebo. It is possible that the placebo effect elicited under such circumstances may be equally powerful as that of experimental TCM drug. This fact complicates the interpretation of the results.

Chapter 9

Promises and Obstacles in the Conversion of Traditional Chinese Medicine to Healthcare Products

Research of traditional Chinese medicine is complicated because many factors affect the results. Some of these factors are the advantages of Chinese medicine; on the other side, these advantages may become obstacles for Chinese medicine research. For example, the complex chemical composition of Chinese herbal medicine is the material basis for multi-target therapy, but the complex chemical composition can cause great difficulties in the active ingredient identification and quality control. Proper use of these factors can greatly promote the development of Chinese medicine; otherwise, they may impede its development. The ultimate goals of research on Chinese Medicine are to relief patients and promote their health. For this aim, marketing and commercialization of herbal medicine is important in production of effective and safe drug and monitoring the post-marketing safety should be also an important part of the research and development of traditional Chinese medicine.

9.1 Multi-target and systems biology

The theoretical system of traditional Chinese medicine emphasizes holistic approach. This concept is similar to the systems biology. Systems biology is a new subject in the field of life science which focuses on all the constituents (genes, mRNA, protein, etc.) in a biological system and the interactions between these constituents under special conditions (1, 2). More and more studies have shown that Chinese medicine contains

the active ingredients. These ingredients have therapeutic effects through acting on multiple targets, which is, in view of systems biology, considered as multi-target drug therapy. The investigation of multi-level, multi-target effects may lead an important research direction in the mechanism study of traditional Chinese medicine. There is much in common between systems biology and the holistic concept of TCM. Therefore, study methods of systems biology have been paid increased attention in present TCM research.

The disease spectrum is experiencing a remarkable change since late 20th century, evolving from single-factor diseases (such as tuberculosis) to multi-factor fashion (such as cancer, cardiovascular diseases). Western medicine makes extensive use of drugs that consist of single chemical compounds. This follows the principle of seeking the "silver bullet" that will act on a single target (3). The Chinese medicine makes use of medicinal herbs. Usually the herbal formulae contain from 3 to 25 different herbs and act on multi-target (4). Disease is caused by imbalance of homeostasis that involves more than a single function of the body or a particular organ. The treatment should be multi-factorial. Chinese medicine does not directly target against a symptom or pathology, but emphasizes the maintenance of harmony of body (5).

In the 21st century, modernization of Traditional Chinese Medicine is facing new challenges. The emerging of systems biology with its new ideas and research methods coincides with Traditional Chinese Medicine. For long time, ancient Traditional Chinese Medicine has been used simply and intuitively as a primary means of cognition without deductive analytical methods, it is unable to collect accurate and objective qualitative and quantitative parameters that could reflect the most essential

feature of Traditional Chinese Medicine. This is the bottleneck in development of Chinese medicine. Several constituents with different pharmacological targets are involved in the therapeutic action of herbal preparation. This characteristic may be an advantage compared to single isolated compounds, especially when the underlying disease has a multi-factorial etiology that is responsible for chronic illnesses. It is a fact that complex herbal extracts have been shown to be therapeutically active and safe (6, 7).

There are two major characteristics of Chinese medicine: one is the holistic concept (整體觀) that emphasizes the unity of man and nature. Under the physiological and pathological conditions, human body works as an organic whole, and the various parts of human body are associated with each other. Another characteristics of Chinese medicine is the *treatment based on syndrome differentiation* (辯證論治). Traditional Chinese Medicine has noted the two sides of a disease in holistic and individual. However, these features are not properly interpreted using the language of modern sciences, so it is not accepted by mainstream medicine. Systems biology reflects an integrated ideal and a tendency of traditional biology to "modern biology". It is the sublimation of the "formation" to "function". (8). Systems biology is similar to Traditional Chinese Medicine in the understanding and treatment of diseases.

As a complex system, Chinese herbs are mostly used in combination. It acts on multiple targets that involve multiple genes and cells to regulate the body's overall balance and homeostasis. In the current drug development model, the active ingredient selected for specific target usually has unexpected side effects.

Western medical researchers often use the method of disassembling prescription to investigate the Chinese herbal formula to look for the specific active ingredient. This method can not reflect the principle of traditional Chinese herbal combination that

normally includes four different components Monarch (君 principal), Minister (臣 adjuvant), Assistant (佐 auxiliary) and Guide (使 conductor), respectively.

In order to maintain the vitality, Traditional Chinese Medicine can not just lie on the "holistic concept", and can not just stop at the level of purifying active ingredients, it should make use of modern advanced bio-medical research methods to upgrade the research methods for Chinese medicine.

Systems biology is unlike conventional biology that is based on the theory of reductionism. Conventional biology is only related to individual gene or protein; it obliterates the linkage between the various parts. Systems biology aims to increase the understanding of biologic systems by looking at the interactions between hundreds of genes, proteins, and metabolites simultaneously (9,10).

Systematic approach already existed for several thousands of years in the practice of Traditional Chinese Medicine, but the underlying theory was not yet understood from a biochemical basis. In Chinese medicine theory, diseases were called syndromes (*Zheng*), which were adjudged based on patterns of various symptoms expressed by the entire body (11). Chinese herbal medicine is complicated in composition, whatever it is a single herb or a compound herbal formula. Various elements of the herbal drug acted on the various targets to induce complex pharmacological effects. Systems biology could be a bridge between Traditional Chinese Medicine and modern medicine (12). If Chinese medicine integrated with systems biology, it would be possible to systemically interpret that traditional Chinese medicine balances multi-target, treatment mechanisms and molecular mechanisms, and provide new approach for traditional Chinese medicine research.

An effective herbal formula organizes the concerted actions derived from different herbs to create holistic, multi-target, multi-dimensional pharmacological actions and achieve effective therapy. Such an approach is not yet embedded in Western medicine,

but the advance of systems biology has created the building blocks to bridge the Chinese medicine approach with modern medicine (12).

9.2 Chemical complexity of herbs—Synergistic effects

Plants contain several hundred constituents (13). Herbal products derived from multiple or even single plants are complex mixtures of numerous chemical entities. Some of them are present in very high concentrations and some very low. In spite of the advanced chemical analytical techniques available, only a small fraction of the constituents have been isolated and identified. The entire chemical composition of herbal preparation cannot be completely defined, nor all active ingredients identified.

The conventional pharmaceutical development is targeted at isolating single component from medicinal plant that can later be manufactured, synthesized or extracted on a large scale (e.g., taxol from Taxus baccata for anticancer therapy).

Complex Chinese prescription is composed of many herbs. There are many problems associated with complex composition. Each herb comprises tens of compounds. These compounds may have further complex interactions (synergism or antagonism) after being taken into the body.

At present, new chemical drug development is confronting difficulties due to the cost and time-consuming. The number of new chemical entities being applied for new drug application is fewer. The safety and effectiveness of many traditional Chinese herbal prescriptions and herbs are reliable.

Chinese herbal formulae are usually composed of 3—25 herbs (4). Synergy is an important issue in Chinese herb medicine. A well example of synergy is the study of

Synergetic effect of Danshen and Gegen in DG (7: 3) compound formula conducted by Institute of Chinese Medicine, The Chinese University of Hong Kong. **Figure 9-1** showed the comparison of the vasodilative effect among DG (7:3) compound formula, Danshen and Gegen. For the dose 3mg/ml, the rate of vasodilation of DG (7:3) compound formula, Danshen and Gegen were 5.3%,min^{-1}, 3.8%min^{-1} and 2.7%min^{-1} respectively where the potency were DG (7:3) compound formula, Danshen and Gegen in descending order. The figure suggests Danshen and Gegen have synergistic effect since DG (7:3) had greater vasodilative effect than the individual herbal effect. (14, 15).

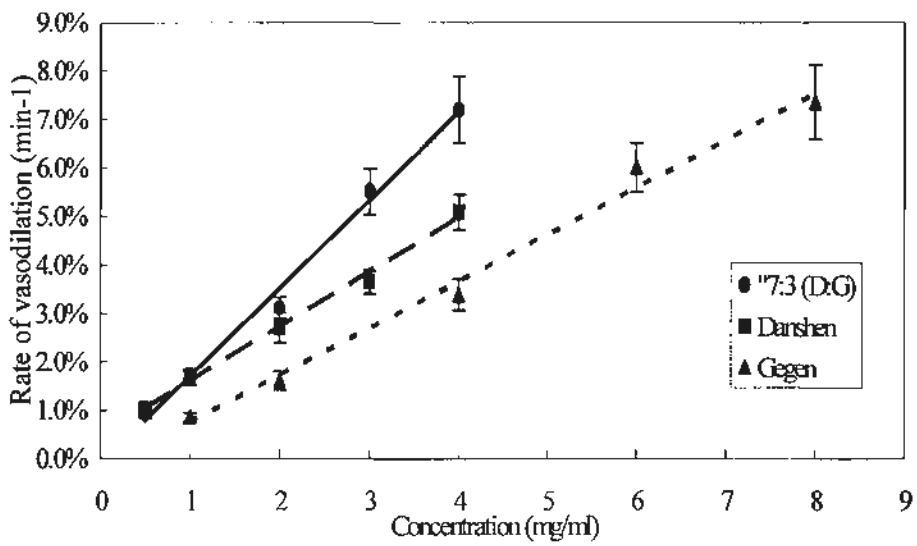


Figure 9-1 Synergetic effect of *Danshen* and *Gegen* in DG (7: 3) compound formula

The ultimate quality criterion of medicinal herbs is the presence of chemical constituents that confer health benefits. For most medicinal herbs, a range of compounds have been ascribed with pharmacological activity and synergistic effects. These active constituents are often complex labile compounds that traditional analytical techniques had difficulty in extracting and quantifying. It is only since the advent of HPLC could be given to setting quality standards based on levels of active constituents or marker compounds that can signify the presence.

Many medicinal herbs have not been subjected to rigid clinical trials but rely on evidence from traditional application. Ingestion of selected ingredients extracted from herbs at elevated dosage may induce unknown side effects. An herbal product may lose synergistic benefits if only part of the ingredients were used, actually the synergistic effects may exist in the multiple active constituents of the herbal formula.

9.3 Market approval--Herbal product registration

In 1998, China streamlined its centralized regulatory processes for all medical products sold or manufactured in China. The State Food and Drug Administration (SFDA) was organized to formulate and implement relevant regulations.

A new "*Administrative Measures for Drug Registration*" was issued on February 28, 2005, and implemented on May 1, 2005. The past experiences suggested that SFDA imposes more rigorous screening on Investigational New Drug (IND) applications which might take very long time to approve. Despite this relatively lengthy IND application process, the new drug approval procedure in China takes about five to eight years to complete, in contrast to an average of eight to ten years in the U.S.

The followings are the key steps to getting a drug approved in China:

- Investigational New Drug applications: a list of application documents (**Table 9-1**) and a sample of the drug should be submitted to the SFDA at the provincial level. Overseas pharmaceutical manufacturers (including Hong Kong) may apply direct to the central SFDA.
- Preclinical and clinical trials: China introduced its first GCP guidelines in 1998; multiple designs, including single- or double-blinded randomized control are allowed. Trials in China are generally not placebo-based; instead, they compare the drug's performance with existing methods of treatment. In 2000, SFDA issued new rules

stipulating that at least 50% of the work for Phase I-III clinical trials must be conducted by accredited National Clinical Trial Centers.

- Quality Testing: once clinical trials have been completed, the manufacturer should provide enough product samples, which have to be manufactured in three consecutive batches, for random tests.
- Certification: a registration certificate is issued once the quality test process has been completed successfully.
- Post-market Surveillance: the level and quality of post-market surveillance activity is rising in China; all of the phase IV trials must be carried out at the same site as earlier trials.

In order to get drug authority approval, selecting proper topics for research & development is very important. The selected topics should be targeted, and should be the areas that Western medicine is unable to resolve:

Allergies—Atopic dermatitis, asthma

Viral infection—Influenza, hepatitis, AIDS

Autoimmune diseases—Rheumatologic conditions

Chronic problems—Pain, mental disorders

Cancers—Adverse drug effects, later stages and recurrences

Another consideration is the clinical indications should be as simple as possible. Although the herbal medicine and traditional Chinese herbal formula have the characteristics of multi-functional claims, the claim of "cure all diseases" herbal preparation always faces a lot of challenges in the process of new drug application. In fact, the more the therapeutic claims, the more work load the drug research, and the more difficult the research process.

In clinical research, a standardized clinical trial is helpful in getting authority approval. The requirements of clinical trials for herbal drug or Chinese herbal medicine remained high. At the same time, the consistency of the quality, stability and efficacy of Chinese herbal medicine or herbal drug should be ensured.

Although it is possible to initiate expanded clinical trials on some well-characterized and widely used botanical preparations without the support of non-clinical toxicity data, additional animal studies may be needed for final marketing approval (16, 17).

Registration of herbal drug and functional food in China (藥品和保健食品在中國的註冊申請)

On October 30, 2002, China's State Food and Drug Administration (SFDA) issued the "*Management of Drug Registration*" (Trial), and it was put into effective on December 1 the same year. Compared with the old management guideline, the new drug registration management guideline is more reasonable and practical, and is beneficial for international market.

According to SFDA, functional food should have three features: 1) should be a food; 2) should have certain function(s); 3) should be non-drug. In addition, the dietary supplements are included in the management of the functional food category, such as vitamins and minerals. They are used to supplement the nutrients and can be registered as functional food.

By the year 2005 the Ministry of Health's regulation "*Health Food Management Measures*" (《保健食品管理辦法》) registered food were not allowed to declare a new function outside the appointed range, a single product should not claim more than two functions and additional functions are not allowed to add after registration approval.

Over the past 10 years, China has approved over 4000 functional foods, however only 22 functions were allowed to claim. In 2003 the functional claims were extended to 27. These restrictions impeded the development of functional food.

The registration procedures of herbal medicine are different from functional food.

In China, domestic produced herbal medicines are divided into nine categories:

1. effective ingredient and its preparation isolated from plants, animals and mineral
2. newly-discovered medicinal natural material
3. substitute for raw material of TCM
4. newly-discovered medicinal part of TCM herb
5. effective fraction and its preparation isolated from plants, animals and mineral
6. Preparations of Chinese herbal medicine, natural herbs
7. changing administrative route
8. changing dose form
9. generic drug

Different type of Chinese herbal medicine, the registration requirements are different. Table 10-1 showed the general requirements of herbal medicine registration application.

Table 9-1 Required documents for Chinese medicine, natural medicine registration application [1]

分類 Categories	內容 Contents
General description and review	1 the drug name 2 relevant documents (patent, GMP, and so on 3 the rationale of developing the TCM drug 4 summary of the research work on the drug 5 a proposal direction (draft) for clinical use 6 a draft of package and label
Pharmacy study materials	7 summary of CMC data

	8 source of TCM raw material and its identification 9 habitat ecology, appearance, growing technique, preliminary processing and processing method 10 morphological and anatomical description, physico-chemical identification (method, data, photograph, conclusion)) 11 specimen of raw material (plant, mineral) 12 manufacturing process and its research data 13 chemical identification of a compound or a fraction) 14 quality control tests and their results 15 quality standard proposal of drug substances and its final product for clinical investigation 16 sample of a final product and a test report on its quality standards 17 stability tests 18 rational of selecting package material and container that contact directly the drug
Pharmacology/toxicology study materials	19 summary of pharmacol & toxicol. 20 pharmacodynamics 21 safety pharmacology (general phar.) 22 acute toxicity 23 chrinic (repeated dose) toxicity 24 allergy, hemolysis, and local irritating tests 25 mutagenic tests 26 reproduction toxicity 27 carcinogenic tests 28 pharmacokinatics
Clinical study materials	29 summary of the clinical work on the drug 30 protocol of clinical study design 31 Direction for clinical investigators 32 a draft of the consent form and the certificate approved by a ethical committee 33 a report on the clinical studies

The drugs that received SFDA approval in China for marketing are increasing. However, this does not imply that the drug registration is easy. On the contrary, from the new enactment of the "*Drug Registration Regulations*" (2007) we can see that

SFDA has gradually increased requirements for drug evaluation. Pharmaceutical industry is a huge market, especially potential markets like China, is growing every year. So far, there are 1800 multinational companies set up joint ventures and invested 2 billion U.S. dollars in China (18).

9.4 The development of Chinese medicine and registration system

In July 1999, *the Chinese Medicine Ordinance of Hong Kong* 《中醫藥條例》 was passed. In December 19, 2003, Hong Kong SAR Government announced that the "*proprietary Chinese medicine registration system*"《中成藥註冊制度》was formally implemented. In theory, the market chaos of proprietary Chinese medicines in Hong Kong was ended. Hong Kong's pharmaceutical market is quite small and not the ultimate goal for Hong Kong's Chinese medicine manufacturers. Entering the Mainland market and overseas markets are the final purpose of Hong Kong's TCM industry.

Constraint factors in development of Chinese medicine in Hong Kong

Traditional Chinese herbal medicine made by Japan and Korea are popular in the global market; the main reason is that they have established a set of standards for scientific validation and registration systems that are well recognized by international pharmaceutical market. Hong Kong has also set similar standards and registration systems; however, the real effective day is still unknown, which affects the desires of manufacturers to produce better herbal products. It might impede the development of Chinese medicine in Hong Kong.

In order to expand the market, many TCM manufacturers in Hong Kong target the

Mainland China market. They want to sell their herbal products in China. What are the basic requirements for herbal drug registration in China? The imported drugs that were initially produced and marketed for registration application in China must meet the following two conditions: 1) the products must be produced in the countries / regions where the manufacturer located and approved by the local authorities; 2) the manufacturers must comply with the quality standards that the host country issued as well as Chinese GMP requirements. These requirements are applied to the drug manufacturers in Taiwan, Hong Kong and Macao.

From the status of Chinese medicine product registration to see the development of Chinese medicine in Hong Kong

The drug safety is the first priority that authorities have to consider when evaluation of drug registration. Only when GMP, GCP and GLP requirements are complied, the safety and efficacy of traditional Chinese medicine can be guaranteed.

Efficacy is fundamental and crucial when Chinese herbal medicinal products are put on the global pharmaceutical market. After very long-term clinical application, the efficacy of traditional Chinese medicine in general is viewed positively. The question is whether the evaluation methodology and efficacy evidences of Chinese medicine are convincing and accepted by the authorities or not. Registration of Chinese herbal products is essential for the development of Chinese medicine because it is the key to obtain the permission of market entrance.

Requirements of proprietary Chinese medicines registration in Hong Kong

Taking into account the formulation, usage history and indications, proprietary

Chinese medicines (pCm) is divided into three categories: 1) established medicines, 2) non-established medicines and 3) new medicines. "Health-preserving medicines" and "Other medicines" are the two sub-categories under the "Non-established medicines category". The "Other medicines category" includes "Single Chinese medicine granules" that fall within the definition of proprietary Chinese medicines (pCm) (19).

Depending on the category of the pCm, the applicant is required to submit different levels of documents in order to prove the safety, quality and efficacy of the product For example, for established medicines, references from Chinese medicines bibliography, Pharmacopoeia or any other National Standards of the People's Republic of China would be sufficient for product efficacy. For new medicines, reports on product efficacy and clinical trials are required.

Established medicines category:

Except for Chinese medicine injections, pCm that is formulated according to any of the following prescription would be regarded as "Established medicines":

1) an ancient prescription (which has been documented in Chinese medicines bibliography in, or before, the *Qing* dynasty);
2) a modified ancient prescription (the prescription of which is based on an ancient prescription with reasonable and rational modifications;
3) a pharmacopoeia prescription (which has been documented in the Pharmacopoeia of the People's Republic of China); and
4) any other prescriptions originated from the *National Drug Standards* of the People's Republic of China and accepted by the Chinese Medicines Board.

The original dose form of the prescription should not be changed; otherwise the pCm will be regarded as "*New medicines category*" (except for those ancient prescriptions provided that their principal manufacturing method remains unchanged). An herbal product made from single Chinese herb and its claimed indications and functions same as its crude drug (except single Chinese medicine granules) is also regarded as established medicines.

Non-established medicines category:

Except for Chinese medicine injections, any pCms, which are used for the purpose of regulating the functional states of the human body, shall be regarded as "*Health-preserving medicines*" in the "*Non-established medicines*" category. The prescription of the "*Health-preserving medicines*" should not contain any newly discovered Chinese herb, new medicinal part(s) of Chinese herb, active group extracted from Chinese herb or set of active groups extracted from compound prescription. Otherwise, the pCm will be required for registration under the "New medicines" category. "Single Chinese medicine granules" that their claimed indications and functions are the same as those of their crude drugs will be regarded as "*Non-established medicines*".

New medicines category:

The prescription that contains newly discovered Chinese herb, new medicinal part of a Chinese herb, active group extracted from Chinese herb, a set of active groups extracted from a compound prescription, Chinese medicine injection, a new Chinese medicine prescription, altered route of administration, new indication or altered dose form willl be regarded as "*New medicines*".

Registration groups of Proprietary Chinese Medicines (pCms)

The registration groups of pCm are *Group I, Group II* and *Group III*. Different registration groups have different registration requirements, and hence require different documents. For pCms under the "Established medicines category" and "Non-established medicines category", applicants may choose to apply for registration in any of the three groups. However, for pCms in the "*New medicines category*", they must be registered according to Group III registration requirements.(**Table 9-2**)

Table 9-2 Documents required for transitional registration of pCms

✓ Required × Not required

Part I: To be submitted upon application	Group I	Group II	Group III
1 Completed Application Form and "Transitional Registration Checklist (A)"	✓	✓	✓
2 Application fee	✓	✓	✓
3 Personal information of the person-in-charge of the company	✓	✓	✓
4. Documentary proofs of manufacture or sales history of the product	✓	✓	✓
5 Product sample and prototype sales pack	✓	✓	✓
6 Master formula	✓	✓	✓
Part II: To be submitted within 1 year from the deadline of application for transitional registration	**Group I**	**Group II**	**Group III**
1 Transitional registration checklist (B)	✓	✓	✓
2. Copy of manufacturing authorization issued by the country of origin (if applicable)	✓	✓	✓
3 Copy of free sale documentation issued by the country of origin (if applicable)	✓	✓	✓
4 Labels and package insert which have complied with the laws	✓	✓	✓
5 Heavy metals and toxic element test report	✓	✓	✓
6 Pesticide residues test report	✓	✓	✓
7 Microbial limit test report	✓	✓	✓
8 Interpretation and principle of formulating a prescription	✓(1)	✓(1)	✓(1)
9 Reference materials on product efficacy	✓	✓	✓
10. Manufacturing method	✓	✓	✓
11. Physicochemical properties of crude drugs	✓	✓	✓
Part III: To be submitted within 3 years from the deadline of application for transitional registration	**Group I**	**Group II**	**Group III**
1 Transitional registration checklist (C)	✓	✓	✓
2. Acute toxicity test report	×	✓	✓
3 Long-term toxicity test report	×	✓	✓
4 Local toxicity test report	×	✓(2)	✓(2)
5 Mutagenicity test report	×	✓(3)	✓(4)
6 Carcinogenicity test report	×	✓(5)	✓(6)
7 Reproductive and development toxicity test report	×	✓(7)	✓(8)
8 Summary report on product safety documents	✓	✓	✓
9 Principal pharmacodynamic studies report	×	×	✓
10. General pharmacological studies report	×	×	✓(9)
11. Clinical trial protocol and summary report	×	×	✓
12. Summary report on product efficacy documents	✓	✓	✓
13. Product specification, method and certificate of analysis	✓	✓	✓
14. Accelerated stability test report or general stability test report	✓(10)	✓(10)	✓(10)
15 Real-time stability test report	×	✓	✓

The followings are the main requirements for registration in Hong Kong:

Product Safety Documents

- Heavy metals and toxic element test: Required to submit the relevant test methods and test reports. If the prescription contains no heavy metals or toxic mineral elements in medicine, then the content of heavy metals and toxic elements should not

exceed the permitted level (**Table 9-3**)

Table 9-3 Permitted levels of heavy metals and toxic element

Heavy metals or toxic element	Maximum Permitted Level (total intake)
Arsenic	1,500 microgram/day
Cadmium	3,500 microgram dose
Lead	179 microgram/day
Mercury	36 microgram day

Non-natural medicines categories of health products are generally not use heavy metals or toxic mineral elements in medicinal herbs.

- Pesticide residue test reports: refer to organochlorine pesticide residues testing. Required to submit the relevant test methods and test reports. Amount should not exceed the maximum residue limits (**Table 9-4**)

Table 9-4 Maximum permitted levels of pesticide residues in the finished products or individual crude drugs of pCms

English name of pesticide	Chinese name of pesticide	Test parameters	Maximum permitted level (mg/kg)
1) Aldrin & Dieldrin	艾氏劑 艾氏劑	Sum of Aldrin and Dieldrin	0.05
2) Chlordane	氯丹	Sum of cis-chlordane, *trans-chlordane*, and oxychlordane	0.05
3) DDT	滴滴涕	Sum of *p,p'*-DDT, o,p-DDT, *p,p'*-DDE and p,p'-TDE	1.0
4) Endrin	異狄氏劑	Endrin	0.05
5) Heptachlor	七氯	Sum of Heptachlor and Heptachlor epoxide	0.05
6) Hexachlorobenzene	六氯苯	Hexachlorobenzene	0.1
7) Hexachlorocyclohexane	六氯環己烷	Sum of its isomers, except for the Lindane	0.3
8) Lindane	林丹	Lindane	0.6
9) Quintozene	五氯硝基苯	Sum of Quintozene, Pentachloroaniline and Methyl pentachlorophenyl sulphide	1.0

- Microbial limit test report: Microbial limits are divided into total aerobic count, mold and yeast count, and specified bacteria (i.e. Escherichia coli,

Staphylococcus aureus, Pseudomonas aeruginosa) number three projects). Test results should be consistent with the microbial limits for various dose forms.

- Acute toxicity test report: The acute toxicity test is to provide a preliminary evaluation on the safety of the drug. The test report should include test methods, results, conclusion and summary and other relevant information.

- Long-term toxicity test report: The long-term toxicity test is to observe the toxic reaction of the tested animals after continuous administration of the test medicine, including their first symptoms & signs and seriousness of intoxication, the development and recovery of tissue damage and organ dysfunctions after the administration is ceased, so as to provide reference for determining a safe clinical dose level. The test report should include test methods, results, conclusion and summary and other relevant information.

- Local Toxicity Test Report: The local toxicity test is to determine any irritation and allergic reactions caused by the pCm. The test report should include test methods, results, conclusion and summary and other relevant information.

- Mutagenicity test report: the mutagenicity test is to examine whether the test medicine has any possible carcinogenicity or reproductive toxicity. The test report should include the test methods, results and conclusions, etc.

- Carcinogenicity test report: Carcinogenicity test is to determine the potential carcinogenicity or tumorigenicity of the test medicine and its metabolites. The test report should include the test methods, results and conclusions, etc.

- Reproductive toxicity test report: The reproductive and development toxicity test is to examine whether the test medicine has toxic effects on animal's reproductivity and whether it has teratogenic effect on their offspring. The test report should include the test methods, results and conclusions, etc.

Safety data summary report: the summary report on product safety documents is to give an overall conclusion and a reasonable assessment on the safety of the pCm.

Product efficacy documents

Interpretation and principle of formulating a prescription: The contents should include an analysis based on the theory of Chinese medicine, (e.g. the different roles of 'the principal (君), assistant (臣), adjuvant (佐) and guiding (使) drugs' in the prescription), the description on the properties and flavours, channel tropism, functions, indications, compatibility and other related information of each drug in the prescription, and the clinical application of this prescription should be analyzed. Prescription sources (i.e. source prescription), prescription form (including Medicine Smell name, dosage, processing methods), usage, dosage, functional indications, and precautions, etc. are also required.

Reference materials on product efficacy: Different categories have different requirements for registration. For Established medicines category, ancient medicine, or any other national drug standards photocopy of the relevant information are required. For Health-preserving medicines in the Non-established medicines category: The claimed therapeutic functions would be supported by research studies, or the function of which have been described in health care literatures compiled by Chinese medicines professionals. For Single Chinese medicine granules in the Non-established medicines category, copies of relevant materials from Chinese medicines bibliography or Pharmacopoeia are required. For New medicines category, pharmacopoeia in ancient books, or to submit a photocopy of the relevant information are necessary.

Principal pharmacodynamic studies report: The principal pharmacodynamic study of

pCms is, through application of modern scientific methods e.g. to develop animal models for selected syndromes or diseases, to preliminarily verify the therapeutic effects and to determine the potency, extent of effects and properties of the pCm. The report should include the test methods, results and conclusions, etc.

General pharmacological studies report: The general pharmacological studies is to observe and identify other pharmacological effects in addition to the principal therapeutic actions of pCms on animals. The report should include the test methods, results and conclusions, etc.

Clinical trial protocol and summary report: Clinical trial refers to trial with humans as test participants. Under controlled conditions, the safety and efficacy of the product are examined and assessed in a scientific manner. Clinical trial is divided into Phase I, II, III and IV. The applicant should submit the clinical trial protocol of all phases, an approval letter from the Ethics Committee, and the summary report of Phases I, II & III of the clinical trial. Within 2 years after the registration of the pCm, the applicant should submit to the Chinese Medicines Board the Phase IV summary report.

Summary report on product efficacy documents: The applicant should draw the conclusion based on the product efficacy documents (e.g. the interpretation and principle of formulating a prescription, reference materials for product efficacy, and the clinical trial protocol and summary report) to give an overall conclusion and a reasonable assessment of product efficacy.

Product quality documents

Manufacturing method: the manufacturing method should include concise

manufacturing procedures based on each processing step, preparation and processing method for each raw herb, as well as the names and quantities of all excipients used. For any procedure that can affect the quality of the finished product, a related controlling method should be specified e.g. number of hours and times required to boil the Chinese herbs. Such as the prescription containing Asarum, the applicant should only use the species that is allowed to use, and only the part of root can be used. Water extraction should be used to avoid the use of organic solvents as the extraction solvent. It is also advisable not to use raw powder directly added in orally taken medicine.

Physicochemical properties of crude drugs: If the pCm contains i) a newly-discovered Chinese herb; ii) a new medicinal part of Chinese herb; iii) an active group extracted from Chinese herb; or iv) a set of active groups extracted from compound precription; then the applicant should submit relevant bibliography or scientific research reports detailing the physicochemical properties of the crude drug(s) of the pCm. Physicochemical properties of crude drugs generally refer to 4 aspects: 'description', 'identification', 'inspection' and 'assay', as well as to other relevant information. If the crude drug(s) of the pCm do(es) not fall into any of the four categories mentioned above, generally speaking, the applicant is only required to submit a copy of the bibliography on the physicochemical properties of each crude drug from references and specify the sources of the relating documents, for example, copy of the monograph recorded in the Pharmacopoeia of People's Republic of China containing description, identification, inspection and assay of the herb, without conducting the relevant scientific researches.

Product specification, method and certificate of analysis: The applicant should submit

the product specification issued by the manufacturer of the pCm. The product specification of pCms should include at least these 4 aspects: 'description', 'identification', 'assay' and 'inspection'. The test results must comply with the requirements for the registration. For example, medicinal products containing Asarum the quality standards must involve the inspection methods and limitation for aristolochic acid I, and the guideline requests that "not aristolochic acid I " is detected, and the inspection report of aristolochic acid I should be submitted.

Accelerated stability test report: Accelerated stability test refers to the regular assessment on changes in product quality to establish the stability and shelf life of the product. The testing product is kept in a specified temperature (37-40 ℃) and relative humidity (75% ± 5%) storage conditions. The test report should include relevant information, test methods, tests for such dose form, summary report on stability tests) ◦ The shelf life deduced from this test should not be longer than 2 years.

Real-time stability test report: Real-time stability test refers to the regular assessment on changes in product quality to determine the stability and shelf life of the product when its sales packaging is kept in ambient conditions. The report on this test should include relevant information on the shelf life of the pCm as proposed by the manufacturer, the test method, tests for such dose form, summary report on stability tests, etc.

General stability test report: The testing product for general stability test should be kept under the storage condition of room temperature (25 ± 2 ℃) and relative humidity (60% ± 5%) for regular assessment on the testing product to provide a basis for determining the shelf life. The report should include relevant information on the

shelf life of the pCm as proposed by the manufacturer, the test method, tests for such dose form, summary report on stability tests, etc. The proposed shelf life of the pCm should not be longer than 4 years.

Implementation of registration system and the prospect of Chinese medicine

The features of Hong Kong Pharmaceutical industries are small scale, about 70% TCM manufactures employed less than 10 employees. Some of pharmaceutical companies locate at small residential buildings. Most pharmaceutical manufactures are still using traditional manufacturing equipments and methods. This is mainly due to small-scale manufacturers are short of financial support and knowledge to upgrade plant equipments to meet the international quality standards for pharmaceutical manufacturing such as Good Manufacturing Practice (GMP). Manufacturers are often lack of understanding of the concept of high quality pharmaceutical manufacturing requirements, so that they are not willing to invest in the TCM manufacture. These TCM manufacturers in general are lack of financial support and knowledge to conduct research and development. The recent TCM products are not validated by rigorous designed efficacy and safety testing, manufacturers only focus on marketing promotion through high-price employed pop stars. If this trend continues, the prospects would be very serious.

Although Hong Kong's academic researchers have ability to perform in-depth investigates, the results are only used for publication, not much use for industry. The real needs of industry are a set of practical documents for successful registration which include quality, safety and efficacy of the TCM product.

The cost of completion of registration tests and reports for TCM product are at least 6

million, and lasts for 3-4 years. Compared with the development of western medicine, it is not expensive. The point is whether HK Government is determined to enforce the policy. This is indeed the only way leading TCM product to the market.

If further development of TCM in Hong Kong is expected, the TCM registration policy should be implemented as early as possible. Domestically it can stimulate TCM firms invest more in R & D, Externally it can help to open mainland China or overseas markets. At the same time, it should be as early as possible to select proper teaching hospitals in Hong Kong as register base for TCM clinical research authorized by SFDA. This will greatly facilitate Chinese medicine manufacturers in Hong Kong for entering Mainland China market. Finally, GLP standard laboratories should be established for conducting recognized pre-clinical safety testing.

9.5 Commercialization of Chinese herbal medicine –from laboratory to market

Historically, China has not been an attractive pharmaceutical market. Its domestic pharmaceutical market is tiny—less than 2% of the worldwide market. This situation, however, has been rapidly changed. Among the top pharmaceutical markets worldwide, China moved from being in the number 11 in 1996, to number 7 in 2002 (20).

Drug development process can be divided into six phases:

- Target identification;
- Lead identification;
- Preclinical development;
- Early clinical development;

- Full clinical development; and
- Approval to market.

Favorable conditions for Marketing Traditional Chinese medicine in Europe and American

1) The accelerated pace of social life, the aging of the population structure lead to disease spectrum changes

With the progress and development of human society, and the acceleration of life pace and the aging of the population, the diseases like malnutrition and bacterial infections were gradually replaced by cancer, cardiovascular and cerebrovascular diseases, neuropsychiatric disorders and other "modern civilization diseases". The changes of disease spectrum of human are taking place. For these "modern civilization diseases," Western medicines are sometimes powerless. Therefore, people suffering from these diseases may turn to Chinese medicine or "alternative therapy". It was reported that 70% of Americans receiving western medicine are also receiving "alternative therapy" (21). Some American hospitals, such as the University of California San Francisco Hospital, has provided service of the "alternative medical clinics" to make up the insufficiency of Western medicine (21). Chinese medicine degree programs and research institutions are set in some European and American countries.

2) The rapid rise of "holistic medicine"

Currently around the world, "holistic medicine" is rapidly emerging (22). It emphasizes the human life and health is the results of the harmonization of human body, the nature and society. The past treatment methods were based on bio-medical model that ignored the human's integrity. Human body and the environment are indivisible as a high degree of physical and mental unity. "Holistic medicine" is

consistent with Chinese medicine theory of the "Heaven and Man unity" and "holistic concept ". Treatment methods of holistic medicine are basically same as Chinese medicine including herbal medicine, acupuncture, *qi gong*, bone setting, massage, diet and so on. Thus, the rise of holistic medicine has brought a rare opportunity for Chinese herbal medicines to the world.

3) The trend of "return to nature" and "green consumption"

In recent years, with the germination and emergence consciousness of "return to nature" and "green consumption", herbal medicines become more and more popular. For many chronic diseases, age-related diseases, the rehabilitation of health care, chemical, drug toxicity and drug resistance, as well as an increase in drug-induced disease, the progressive deterioration of the ecological environment and so on, natural medicines or herbal drug therapy seems more suitable to solve the these problems.

1) Traditional Chinese medicine is becoming a source of research and development of new drugs

Statistics showed that pharmaceutical companies in Europe and the United States have to spend 7 to 10 years, 80 to 350 million U.S. dollars, to screen and obtain a drug, such as anti-cancer drug, from 4000 ~ 5000 synthetic chemicals, the screening hit rate is low to 1/20000) (21). The drug resistance and toxicity of pure synthetic single-component drugs has been difficult to resolve. Traditional Chinese Medicine has thousands of glorious history with its profound systematic theory and rich experiences in treatment of diseases, the resources of Chinese medicine are 12,807, of which 11,146 from plants, 1581 from animals, 80 from minerals. Commonly used Chinese herbs are also up to 5 to 6 hundreds, while the herbal formulae / prescriptions formed by these resources are 100,000 (23, 24). According to statistics, the TCM

prescriptions are over 300 thousands, among them, 60 thousands are documented (25). Therefore, there is a pattern for the development of new drugs from traditional Chinese medicine, the advantages of the drug development pattern are high rate of hitting, inexpensive and short cycle. Since 1992, China has developed about 200 new drugs from traditional Chinese medicinal plants)(26) In Europe and the United States, at least 60 new drugs are extracted from natural plants, such as *ephedrine, Harringtonine alkali, paclitaxel, camptothecin.*] (26). At present, many European and American pharmaceutical companies have invested a lot of money to expand their R&D in herbal medicine research; they also make use of traditional literatures to look for valuable formulae. Some pharmaceutical companies have established global centers of plant identification, and collaborated with some domestic research institutions to find new drugs from the treasure-house of Chinese medicine.

2) The restrictions of Chinese medicine used in Europe and the United States have been loosened in the levels of laws and regulations

In recent years, with the popular of Chinese medicine, pharmaceutical industries are very interested in its unique effects, and began to force the governments relax the restrictions on herbal medicine. For example in Germany and France, the government and pharmaceutical industries accept herbal medicine as a alternative medicine for synthetic drugs, there are licenses for herbal products, which can be available in pharmacies as OTC drugs. The costs can be reimbursed. In October 1991, House and Senate of United States approved the establishment of the Office of Alternative Medicine (OAM) to conduct scientific research and evaluation of alternative medicine such as herbs, acupuncture, naturopathy, nutritional therapy, etc. In October 25, 1994 FDA signed into law the *Dietary Supplement Health and Education Act* , DSHEA). According to this law, Chinese medicine is no longer a food additive, but as dietary

supplements between food and drug. In 1997 the U.S. FDA has issued a new guidance "*Application Guideline for Natural Mixture of Medicinal Plant* ", started to accept herbal compound preparation as therapeutic drug. China's herbal products have got FDA approval as Investigational New Drug (IND) for clinical studies. All these events indicated that traditional Chinese medicine as a treatment drugs has drawn attention of the global pharmaceutical industry and authorities, and gradually accepted by the international communities.

Although the international situation of traditional Chinese medicine is encouraging, the status of traditional Chinese medicine in the international pharmaceutical market, is extremely serious. At present, the sales turnover of global herbal products has reached 12 billion U.S. dollars per year, in Europe the sales turnover was about 6 billion U.S. dollars (26). In China, annual export volume of Chinese herbal medicine was only 655 million U.S. dollars, of which 39 million U.S. dollars were sold to European Communities (EC), 41 million U.S. dollars were sold to the United States (27). China is a major source of medicinal plants with unique advantages. However, China's export of traditional Chinese medicine was only 3% in the world's Botanicals trade(28). The harsh reality forced us to calmly analyze the shortcomings and deficiencies.

Unfavorable factors of Chinese herbal medicine to Europe and the United States

1) Social, historical, cultural and lifestyle differences between East and West

As the differences in historical and cultural backgrounds between East and West, philosophy is also very different. Chinese medicinal theories, such as *yin yang* and *five phases* theories, visceral manifestations and symptoms, blood *qi* and meridian, cold and heat syndrome and treatment based on syndrome differentiation, as well as on the nature and flavour, channel tropism, Monarch (principal), Minister (adjuvant),

Assistant (auxiliary) and Guide (conductor) and so on, all these for the Westerners who are used to think in the way of micro-analyzing is extremely difficult to understand. But also in Western science and Western medical theories there are no accurate vocabularies to express the concept of Traditional Chinese Medicine. Therefore, to be understood, accepted, widespread and application of traditional Chinese medicine by the Westerners, there is still a long way to go. This is not just a medical issue; it is related to statutory status. Therefore, Chinese herbal medicine has been excluded from the mainstream medicine by West countries, and only exists in the way of alternative therapy. This makes it difficult for Chinese herbal medicine as a drug to enter the international drug market, it only exists as a dietary supplement or food additive. So it cannot advertise as drug and can not be sold in pharmacies, and not covered by insurance. Even in some countries where restrictions are less, license fees are too high. For example in Germany, the cost for a herbal drug license were up to several million Dutch mark, the duration required for the application was at least five years in general)(27).

2) Export of Chinese herbal medicine facing strong competition from other countries' traditional medicine

Traditional Chinese medicine is one of the world's traditional medicines. In the United States, Europe, Australia and other Asian countries the herbal medicine markets are also shared by traditional herbal medicines from other countries. According to statistics, apart from China, there are about 170 foreign companies and at least 40 research institutions in the world engaging in new drug research and development with natural herbal source〔22〕. For traditional Chinese medicine, Japan's approach is taking "making use of TCM and developing its own system", its purpose is to be the center of traditional medicine. Annual gross domestic value of Japan's "Kampo" has

over 100 billion yen. The output of Tsumura "*Shun Tian Tang*" (津村順天堂) alone was equivalent to total annual export of Chinese medicinal products in China. In the new drug research and development of traditional Chinese medicines, Japan has achieved remarkable results with great market share globally, for example *Kyushin*, Tsumura *Chailing decoction* (津村柴苓湯) etc. Some famous herbal preparations such as *Niuhuang Qingxin Wan*(牛黃清心丸), *Zheng Lu Wan*(正露丸) produced by Japan are also very popular in Europe and USA〔29〕. South Korea's strategy is leading by high-quality products with high-price approach. Korea Ginseng alone has made 175 million U.S. dollars annually, equivalent to 58% of total sales of China's annual export of Chinese herbal medicines, and its price increased by about 12% per year. Today, Korea ginseng's prices is 10 times higher than Chinese cultural ginseng〔27〕. Western pharmaceutical companies have set up the Department of Natural Medicines, and actively develop Chinese herbal products. Ginkgo preparation produced by a German company Schwabe the sales turnover has reached 500 million U.S. dollars〔29〕. Foreign pharmaceutical companies use our ancient prescriptions and medicinal herbs transform into their own scientific preparations and sell back to China to make money.

3) Lack of internationally recognized and accepted quality control standards for domestic proprietary Chinese medicines

Traditional Chinese Medicine in research & development has been gradually conducted according to GCP requirements in clinical research, according to GLP requirements in safety evaluation, and according to GMP requirements in manufacturing process. However, the testing methods and standardization of active ingredient content has not been fully in line with international standards. It is

sometimes reported that heavy metals, pesticide residues, toxic contents, and the quality of packaging materials did not satisfy the requirements. It is difficult for foreign drug authorities and experts to believe the unique efficacy of traditional Chinese medicine if the quality of herbal drugs fails to meet the requirements of Europe and the United States.

At present, China's exports in Chinese medicine are mostly raw herbs, prepared Chinese herbal medicines are less than 30%(22), in which fine dosage form with high-value-added products are rare.

4) Insufficient investment and innovation in Chinese medicine research and development

In recent years, Europe and the United States, Japan and South Korea and other developed countries have invested a lot in research and development of traditional medicine, however the investment of domestic Chinese medicine manufacturers in research and development is far from sufficient, especially in the pharmacological effect evaluation, active ingredient extraction and identification, and new formula innovation. Further more, insufficient intellectual property (IP) protection policies make pharmaceutical industries rely on domestic market without ambitious plan for global markets, R&D repeated in lower level is quite common, which seriously impact on the modernization and internationalization of traditional Chinese medicine.

Strategies of Chinese herbal medicine to market European and American

1) Production of traditional Chinese medicine

The improvement of the quality control of Chinese medicine including preparation of quality control standards and uniformization of testing methods are fundamental. The

collecting and processing of crude herbs at the original cultured site should follow the GAP requirements. In order to ensure the quality, standard operation procedures of crude herb collection and identification should be established. Herbal medicines should be free not only from botanical contaminants, but also from residual pesticides or fumigation agents and from pathogenic micro-organisms or microbial toxins.

Research and development of new dosage forms are required in order to meet the needs of international markets. If Traditional Chinese Medicine wants to be marketed globally, it should be effective, micro dose-oriented, multi-channel delivery and easy to carry and store.

Implementation of the GMP standard to enhance the level of production management of Chinese medicine marks the quality of Chinese herbal product accepted internationally. Failing to satisfy GMP standards, the quality, reliability and uniformity of the product is difficult to be assured, the product can not be recognized by the international market, which will greatly affect the market share of proprietary Chinese medicines in the international markets. The full implementation of GMP is the only way to achieve the modernization of traditional Chinese medicine, which is also a permit for Chinese herbal medicine to enter Europe and the United States.

2) Global Marketing Strategy of Chinese herbal medicine

Considering the conditions and characteristics of the herbal products to explore the feasible way for marketing globally, well understanding and studying of the laws and regulations of registration of various countries is essential. For example, traditional Chinese medicine sold in the U.S. market has five forms that are health food, herbal supplements, dietary supplement, non-prescription drugs (OTC) and prescription drug (NDA). The first three categories are equivalent to functional food in China. For these categories the requirements and investment are relatively low, it is quite easy to

satisfy the standards for Chinese Herbal Medicine. Large-volume export to expand the impact of Chinese medicine in the short term can be resulted. While doctors and patients become familiar with and accept the traditional Chinese medicine, it is beneficial to prepare for future application as medicine. According to FDA regulations, any food that is consumed by large number of populations and confirmed that is a safe and effective product, which is strong evidence for application of prescription or non-prescription drug. For the latter two categories, if the application followed the drug registration procedures for the United States and Europe markets, the registration procedures are extremely complicated and expensive. However, this is the only way for traditional Chinese medicine as drug to the global markets. *Guidance for Industry Botanical Drug Products* (2004) of USA indicated that the registration requirements for botanical drug are close to that of Class 1 and Class 2 new Chinese herbal medicine. The registration data of Chinese Class 1 and Class 2 new herbal medicine used for domestic registration can be used for FDA New Drug Application (NDA) if the registration data were prepared according to the GMP requirements in manufacturing, GLP requirements in safety studies and GCP requirements in clinical trials.

For the potential enterprises, more attention should be paid to GMP, Drug Master File (DMF) preparation and clinical trials, and also to cooperation with consulting companies or enterprises of Europe and North America targeted on several aspects of the above mentioned issues, to improve work efficiency.

3) Research and development of Traditional Chinese medicine and education

For the purpose of putting in force of the modernization of traditional Chinese medicine, we need to select some herbal medications with outstanding efficacy, minimal toxicity, and clear active ingredients, and quality controllable, to perform the

second research and development to confirm its safety, quality and efficacy and then to complement modern medicine. The selected herbal medicine should have the characteristics of Traditional Chinese Medicine, and be able to give full play to traditional Chinese medicine compound in multi-target, multi-level treatment of the body as a whole, and be suitable for the preparation of new dosage forms by using modern technology. In line with international market demands, we should pay more attention to the cooperation with research institutes and consultancy units of various countries and/or regions for well preparation of various documents for registration.

Establishment of Clinical trial base: At present, the Western consumers still doubt the efficacy of herbal medicine. In the present situation, the safety and efficacy of Chinese medicine is the key issues for marketing new products. Without license and related scientific research evidences of Chinese medicine, it is impossible to market globally. Not only we cooperate between the domestic institutes to conduct joint research, but also establish various forms of clinical research centers or groups with abroad hospitals to conduct clinical trials with Chinese herbal medicine. If we could work together with famous institutes in TCM research and have published academic reports conducted by foreign experts and professors to support the efficacy of Chinese medicine, it would be conducive to the promotion of Chinese medicine theory, and also helpful for a new drug application for registration.

Government should carry out the research on quality standards for Chinese herbal medicine according to WHO requirements of "safety, efficacy, stability, consistency, and economy" for traditional medicine, establish quality standards for raw materials, semi-finished and finished products to meet the requirements of domestic and foreign markets to ensure that proprietary Chinese medicines access to international markets

in the form of high quality standards.

9.6 Post-marketing surveillances

Drug safety and post-marketing surveillance have become important public health issues. Limitations of pre-marketing drug studies can result in a failure to predict adverse clinical effects in the post-marketing environment.

The evaluation and surveillance of post-marketed drugs may be difficult because a national monitoring system is needed to set up. The post-marketing safety surveillance for Chinese medicine has its own characteristic. Compared with chemical medicine, the efficacy onset duration of Chinese herbal medicine is longer. In pre-market safety assessment and during clinical studies, some adverse reactions may be difficult to define. Therefore, post-marketing safety monitoring is particularly important in Chinese herbal medicine. The unique characteristic of traditional Chinese medicine is multi-target; it may show various adverse reactions. A comprehensive observation / supervision are needed. At present, China has established a system of post-marketing drug safety monitoring (30).

Chapter 10

Discussion

For 5000 years, Chinese practitioners and scientists had never stopped their pace of research in Chinese medicine. During the long period of time, Traditional Chinese Medicine had experienced brilliance, darkness; rapid development, stagnation and even abolishment; it had been regarded as a panacea, and it also faced criticism. Although recently Chinese medicine is respected again in the world and the enthusiasm for traditional medicine research is renewed, however, there is still no proper way for the research and development of Chinese medicine. We believe that with the progress of science and technology, as well as our understanding of the nature of disease, traditional Chinese medicine would eventually find its position in integration of mainstream medicine, and make great contribution to human health.

10.1 Efficacy-driven approach in Chinese herbal medicine research

Lack of knowledge about the mechanisms and active substances of TCM does not prevent its application. Since most herbs have been used for hundreds of years, they should be more or less reliable. The safety and efficacy are already well documented, but their practical utilization in specific clinical circumstances needs to be further established. We need to acquire an updated understanding on the effectiveness of the herbal preparations on disease entities. That is why we should not be satisfied with records on efficacy alone but should start a series of clinical trials and pre-clinical studies to further prove the efficacy of Chinese herbal medicine (1, 2).

Past experiences on herbal medicine research were very narrow. Research work was mainly confined to the laboratory where active ingredients were being extracted from herbs. Only a few successful examples, like *Qinghao* for malaria, can be quoted. The laboratory-orientated approach had been slow, expensive and unreliable.

The mechanism-centred approach to TCM research is formed partly by the belief that every TCM therapy works and therefore further demonstration of its clinical efficacy is unnecessary.(3). According to our past research experience, we believe that efficacy-oriented approach is a practical way of research in Chinese medicine.

The efficacy-driven approach starts with clinical trials using appropriate herbal formulae in evidence-based randomized controlled trial protocol. When efficacy is proven, the formula used is further authenticated and considered for drug development.

The center of TCM research is the clinical trial that is targeting on an important clinical problem which usually fails to find perfect solution in modern medicine. Choosing an appropriate and acceptable methodology for clinical trials would enable the results of the Chinese medicine to be widely recognized by mainstream medicine. Without clinical efficacy no assumption should be made on observations based on the quality of the herbs used, or laboratory evidences of effective biochemical or biological activities.

Parallel with the clinical trial, in-vitro and in-vivo explorations are carried out in the laboratory to find out the biological mode of action of the herbal formula being used for the clinical trials. Using the results of the laboratory tests, the scientific basis of the clinical efficacy could be worked out.

In the efficacy-driven approach, investigation into the mechanisms and the search for active substances is also important, but should be undertaken after clinical efficacy is

firmly demonstrated.(4). If a therapy does not clinically work, then it should be discarded and not subjected to further investigation. Demonstration of clinical efficacy first will thus save resources by avoiding unnecessary basic research into ineffective therapies that are clinically ineffective, thus sparing precious research resources(5).

The methodology of clinical research utilizing herbs therefore, will be centred on a trial designed of evidence based medicine, while biological and toxicological tests are done in parallel to work out the mechanisms of action and safety, and further supporting work must include sophisticated tests of authentication, which establish the chemical and if necessary, the molecular DNA profiles of the individual herbs being used. (**Fig. 10-2**)

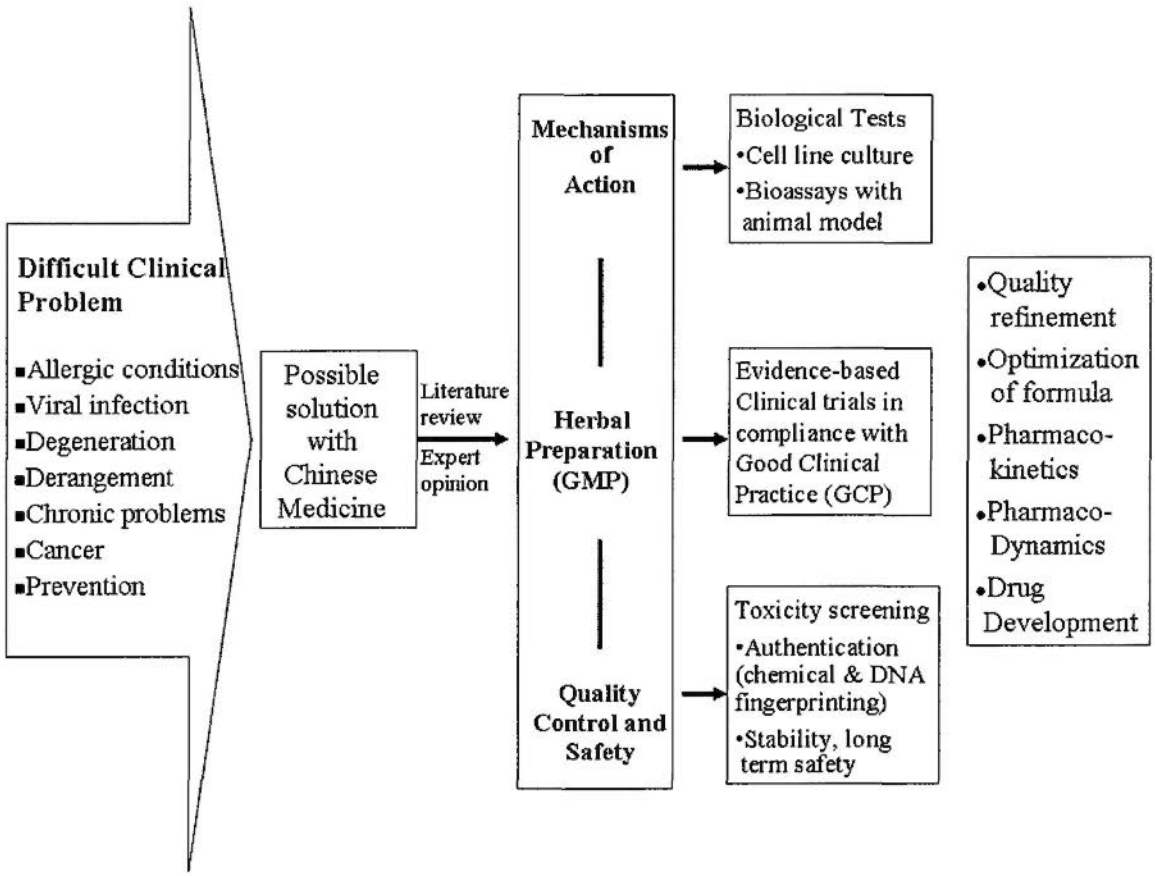


Figure 10-1 The Efficacy Driven Approach, Clinical Trial Leading, Biological Tests & Quality Control in Parallel, Three Prong Approach (6)

10.2 Herbal drug development

There is a great demand for herbal medicines because of their wide biological activities, higher safety margin than the chemicalc drugs and lesser costs. The process of new drug discovery has been estimated to take an average period of 10 years and cost more than 800 million dollars (7, 8). It is estimated that only one in 5000 lead compounds will successfully advance through clinical trials and be approved for use. In addition, research and development of new chemical drug become very difficult, and turning to herbal medicine is an inevitable trend. From above-mentioned procedures we can see that new drug development is a risky, time-consuming project.

The classical pharmaceutical development concept is targeted at isolating one single component from a medicinal plant that can later be manufactured synthetically or extracted on a large scale (e.g., taxol from Taxus baccata for anticancer therapy). Such a product does not fall under the definition of an herbal medicinal product.

In contrast, several constituents with different pharmacological targets are involved in the therapeutic action of herbal medicinal products. This characteristic may be an advantage compared to single isolated compounds, especially when the underlying disease has a multi-factorial etiology that is the case in many chronic illnesses. It is a fact that complex herbal extracts have been shown to be therapeutically active and safe (9, 10).

Herbal drug development includes various steps starting from authentication of raw herbs, correct identification, pharmacognostic and chemical quality standardization, safety and preclinical pharmacology, clinical pharmacology and randomized controlled clinical trial.

Classical ancient Chinese herbal formulae were demonstrated efficacy and safety after many years of clinical use with a solid theoretical and empirical basis. For these

Classical herbal formulations, the secondary development to scientifically and technologically prove the safety and efficacy is critical. Research and development could be conducted as follows: 1) Improving the dosage forms: changing the poor taste, enhancing the contents of active ingredients and reducing the dosage to satisfy the modern lifestyles. 2) Optimizing herbal formulation: clearly know the composition of effective ingredients and pharmacological activity, and the targets and the action mode, has good clinical application prospect and strong market competitiveness, making it easier to enter the international market; 3) Strengthening the study of indication: According to the herbal composition and herbal forming theories to study the new indications, expand the scope of indications, and highlight the best indication, and finally clarify the basic mechanism of action, less clear-cut indications and more targeted herbal medicine would be achieved.

Despite a long history of use with proven efficacy in a variety of pathological conditions, comprehensive validation in terms of biomass authentication, chemical characterization, process development, safety assessment and efficacy evaluation is required to qualify many herbal remedies as drug substances for prescription or OTC use. Solid scientific evidence to the functional claims would be essential to this class of medicine for its acceptance by the mainstream pharmaceutical market.

There are about 12807 kinds of Chinese herbal medicines and botanical medicines in China (11, 12). It is quite possible that new drugs from those resources are explored. During the drug development and research in Chinese medicine, apart from considering the requirements of Good Agriculture Practice (GAP), Good Extracting Practice (GEP), Good Manufacturing Practice (GMP), Good Laboratory Practice (GLP), and Good Clinical Practice (GCP), in-depth study of Chinese herbal medicine to develop new drug like Veregen (sinecatechins) approved as a drug by FDA (13), may be the future trend of drug discovery for Chinese medicine.

10.3 Systems biology and modernization of Chinese herbal medicine

Systems biology, the concept and research method were systematically presented by American professor Leory Hood (14), is a new science that studies the overall level of life-support systems, and is second to genomics, proteomics after first time introduction of the framework of the knowledge of molecular biology (**Fig. 11-1**) (15). Systems biologists believe that it is far from sufficient for such a complex body of life only to study of a single gene. Because the genes in the body are not isolated, the interaction between genes is not a simple linear relationship; it has a complex regulatory network (16). In recent years, new scientific breakthroughs in technology and methods provide a better platform for collaboration with modern science, which is helpful in the development of Chinese medicine. One of the many representatives is the development and application of systems biology.

TCM takes advantage of a specific holistic method to manage the diagnosis and treatments of some diseases. The specific holistic method TCM generally uses is based on the episteme method approach, which derives from the conceptions of unceasing movement and dynamic holism.

Systems biology medicine is increasingly linking Western medicine and Chinese medicine. From the beginning Chinese medicine is a multi-target technology; however the multi-target concept of Traditional Chinese medicine is not built on the molecular level, but on a system of thought or philosophy. The multi-target technology of modern systems biology uses molecular techniques based on a single target technology. Since the modernization of Chinese medicine has been proposed, extraction of active single ingredient from single herb has played a certain role in new drug development, and in elucidation of the action mode of Chinese medicine. With

the further investigation, researchers found that the active ingredient of herbs was isolated, but its efficacy was reduced or even disappeared. The more purified the fewer efficacies. Chinese herbal preparation made by modern technique is often not reaching the therapeutic effect of herbal formula decoction. Modernization of Traditional Chinese Medicine has been in a perplexing status that is simple repetition with low level and slow pace. Chinese herbal medicine is too complex; it is difficult using a period of several decades to achieve the overall understanding at the gene level. While a herbal formula is formed, the active ingredient from single herb is re-combined, it may generate a new active substance or strengthen synergistic effects, or antagonize each other to lower efficacy or reduce toxicity, or increase the side effects, etc., thus a new and higher-level system is formed, a strong pharmaceutical effects may occur when the new active ingredients act on the target systems. The systemic nature of traditional Chinese medicine demonstrates that the efficacy of traditional Chinese medicine basing on active ingredient cannot fully reveal the essential attribute of Chinese herbal medicine.

The characteristic of traditional Chinese medicine is multi-component in composition, multi-channel in action mode, and multi-target in therapy, so that the efficacy evaluation of traditional Chinese medicine should be holistic approach.

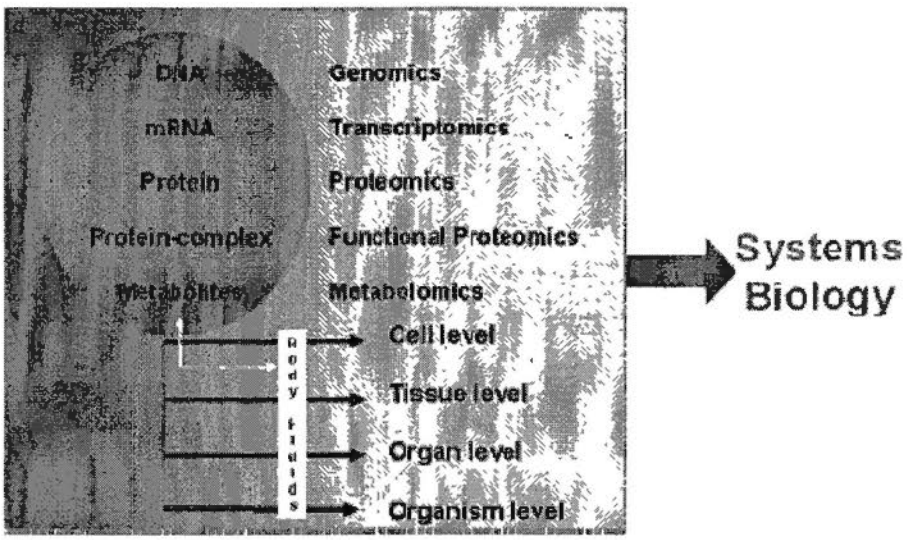


Figure 10-2 The different levels of measurement in a systems approach (17)

Modernization of traditional Chinese medicine (TCM) means the combination of TCM with modern technology, modern academic thoughts, and modern scientific culture, in which the most important point is to elucidate the active component of TCM, especially the material foundation of compound prescriptions and their pharmacodynamic mechanisms. Modernization of TCM not only needs to look at the physical and chemical properties and biological activity of a single herb and herbal formula, but also need to study the reactions between chemical compositions, and the target system of human body and its synergistic action mode.

The holistic thinking and the principles of prescription of Traditional Chinese Medicine and the interlinked nature of systems biology will promote the development of Chinese medicine and theoretical innovation.

In July 2006 the U.S. Food and Drug Administration (FDA) approved an anti-HIV drug named *Atripla.* The drug is composed of three U.S. FDA approved drugs *Viread*, *Emtriva*, and *Sustiva*. They were made together as a combination drug for "cocktail therapy". It was fully demonstrated that the mainstream medicine has also recognized the limitations of monotherapy of Western drug, and began to learn from the model of

Chinese herbal compound.

The introduction of the concept of systems biology, enabling the study of living systems from a holistic perspective based on the profiling of a multitude of biochemical components, opens up a unique and novel opportunity to reinvestigate natural products. In the study of their bioactivity, the necessary reductionistic approach on single active components has been successful in the discovery of new medicines, but at the same time the synergetic effects of components were lost. Systems biology, and especially metabolomics, is the ultimate phenotyping. It opens up the possibility of studying the effect of complex mixtures, such as those used in Traditional Chinese Medicine, in complex biological systems; abridging it with molecular pharmacology. This approach is considered to have the potential to revolutionize natural product research and to advance the development of scientific based herbal medicine.

Chinese herbals are used in formulation to regulate balance and homeostasis of body holistically, acting on multiple targets systems, such as cells and genes. The study of Chinese herbals demands not only the verification of the physicochemical property and bioactivity of every component in a formulation or a single herb, but also study on the reactive laws of different components, the interaction of them with target systems, and the synergistic effect on the body.

Systems biology is a new territory in life science and represents the future of biomedicine in the 21st century. Its potentiality in medicine is infinite and may have significant impact on basic medicine, clinical research and drug development. Integration of systems biology into the study of TCM would make it possible to interpret the essence of TCM syndromes and to explore the molecular mechanism of therapeutic effects of herbal formulation with multiple targets, balanced regulation

and treating both the principal and the secondary aspects of a disease at the same time.

The development of systems biology related disciplines would gradually improve the biotechnological research methodology and philosophy. Systems biology medicine is increasingly linking traditional Chinese medicine and Western medicine. However, in the process of this link there may be a major crisis in modernization of Chinese medicine. Some traditional treasures in Chinese medicine may be lost.

10.4 Statistical significance versus clinical relevance

Statistical analysis in clinical research is used to show that the findings are not likely due to chance. The concept of power of a clinical trial refers to the probability of detecting a difference between study groups when a true difference exists. When comparing two treatment groups, hypothesis testing is widely used. However, investigators should be more interested in statistical methods, which elicit the magnitude of the differences between treatment groups, rather than a simple indication of whether or not the differences are statistically significant. Statistical significance does not necessarily imply clinical relevance. If the true difference between two treatment groups is so small that it is clinically irrelevant, a sample size can be found for which this difference is statistically significant. On the other hand, if the difference between treatment groups is statistically non-significant, it may still be clinically important.

When evaluating the validity of a study, the reader must consider both the clinical and statistical significance of the findings. A study that claims clinical relevance may lack sufficient statistical significance to make a meaningful statement. Conversely, a study

that shows a statistically significant difference in 2 treatment options may lack practicality (18).

Statistical p-value is mysterious; many people put their faith in the success of the study on the p-value. The meaning of p values is that the probability of mistakes of rejecting the null hypothesis, that is the probability of mistakes to give a conclusion for the excellent efficiency, equivalent and non-inferiority. P values are usually <0.05 or <0.01, they are now calculated using statistical software, it should write the exact p-values, so we can better judge the reliability of the conclusions. There is a misunderstanding: the smaller the p value, the greater the difference between the comparison groups. Correct understanding is, the smaller the p value, the more fully the reasons for refusal to hypothesis and the conclusion of "the difference was statistically significant" is more reliable. p value is related to the sample size. The larger the sample size, the smaller the p value.

It is critical to consider the statistical interpretation of results with focus on the clinical significance. It is noteworthy that the statistical results and medical conclusions may present contradiction. When the sample size is large enough, even if the effect of the difference between the comparison groups is very small, there may be statistically significant difference but without any clinical relevance. Statistical conclusion and clinical conclusion may be inconsistent; therefore, we should focus on rigorous scientific design and correct statistical analysis.

When evaluating the validity of a study, the reader must consider both the clinical and statistical significance of the findings. A study that claims clinical relevance may lack sufficient statistical significance to make a meaningful statement. Conversely, a study that shows a statistically significant difference in 2 treatment options may lack

practicality.

A statistically significant difference may be indicative of an important difference or of a trivially small difference detected by a sensitive statistical analysis. A difference which is found to be statistically non-significant may point to a similarity between treatment groups or to an insensitive statistical analysis which cannot detect a possible important difference. Thus, a finding of 'non-significant difference' is for all practical purposes nothing more than a confession of failure to dispose of the null hypothesis (19)

Authors of RCTs usually report statistically significant differences between groups, and conclusions are often based on this statistical significance (20). If $P < 0.05$, the conclusion is usually that the intervention is more effective than the comparison; if $P > 0.05$, the intervention is considered not more effective. This P value is not very informative and only indicates the chance of the observed effect, not considering its size (21). The P value does not indicate if the effect is clinically important.

Positive conclusions should be based on statistical significant and clinically important outcomes. Small studies may show clinically important findings that are not statistically significant. A meta-analysis may resolve this problem of underpowered studies. However, if findings are statistically significant but not clinically important, a meta-analysis will not change the conclusions.

The use of multiple outcomes in the field of low back pain research warrants caution. If the intervention of interest is statistically significantly better than the comparison on one of the outcome measures, the conclusion usually is that the intervention is more

effective than the comparison regardless if there is no difference on any of the other outcome measures. Obviously, one would expect that the results support a prior hypothesis about the potential working mechanisms of the intervention. These mechanisms should a priori lead to the choice of one primary outcome measure. This primary outcome measure should be used to define the expected difference in effect in sample size calculations.

In accordance with the recommendations of Chan et al (20, 22) that may lead to a reduction of the number of studies that exaggerate the results, we would like to suggest that authors: 1) choose one primary outcome measure for a trial, based on a plausible working mechanism of the intervention; 2) perform a sample size calculation; 3) use the minimally clinically important difference (MCID) for the primary outcome measure as expected difference in sample size calculation; 4) report within-group changes and between-group differences; 5) report mean between-group differences with 95% confidence intervals.

Conclusions of trials should not only be based on statistical significance of effects, but also on clinical importance. When systems biology is considered, investigating on one outcome basing on the assumption of one cause and relying on the 0.05 level of one p value is certainly insufficient indication of efficacy value. Before all causes are known and before more targets could be specifically set up, p value could only be one of the relatively reliable indicators of efficacy.

10.5 Synergistic activities of Chinese herbal medicine

The benefits of herbal medicine often come as a result of the synergistic efficacy of

multiple compounds. When each fraction is considered separately, the synergistic effect cannot be shown. Synergy occurs if two or more herbal ingredients mutually enhance each other's effect more significantly than the simple sum of these ingredients (22, 23). There have been reports of the total contents of a herbal product showing a significantly better effect than an equivalent dose of a single isolated active ingredient (22, 24). Some herbal combinations are more effective than the constituent herb used alone (25). Synergistic effects will be lost if the herbal formula is divided into a target driven single element (17).

Most Chinese herbal formulations are composed by several herbs. If two or more herbs are being combined, they can be interacted as synergistic effects: reduced toxicity, enhanced bioavailability, and cumulative effects (26).

Appropriate integration of Chinese and modern medicine may have synergistic effects such that treatment outcome is enhanced and side-effects are suppressed. For example, in the case of tonsillitis, co-administration of *Radix Isatidis* (板藍根) with Trimethoprin (TMP) significantly enhances the immune system, the outcome may be much better than administration of either drug alone. *Flos Lonicerae* (金銀花) increases the effectiveness of penicillin on drug-resistant Staphylococcus aureus; the synergistic interactions of *Rhizoma Coptidis* (黃連), *Cortex Phellodendri* (黃柏) and tetracycline, acheomycin, aspirin, TMP often initiated a better outcome in the treatment of diarrhoea and malaria. The coadministration of *Liquorice* and Streptomycin reduced the toxicity of the latter on the auditory nerve (27).

10.6Characteristics of traditional Chinese medicine research

In scientific research of Chinese medicine, especially clinical research, there are many

difficulties to the research work, which are created due to the characteristics of Chinese medicine itself. WHO pointed out that the acceptance of traditional medicine is due to its clinical efficacy, although the methodology used to evaluate the efficacy may not be the most suitable method.

The treatment of diseases with Traditional Chinese Medicine has gone through the development process from simple to complex, from lower to higher, and from unilateral to compound. Chinese herbal medicine is characterized by multi-component, multi-level action and multi-target, so the research method should be based on various approaches.

Specificity of clinical research

In the study design:

The treatment with Western medicine is aiming for the target and lesions. The treatment with Chinese herbal medicine, especially herbal compound, is holistic approach. The effectiveness of Chinese medicine is not only for the treatment of the disease, but also for the prevention of sub-health status (treatment of undisease), and the rehabilitation. Traditional Chinese Medicine Therapy has unique advantages in practice, but if we just copy the existing evaluation methods for Western medicine, it would be difficult to demonstrate its superiority, or even possible to draw a negative result. Therefore, it is needed to establish the clinical efficacy evaluation method in line with the characteristics of Chinese medicine.

Because Chinese medicine and Western medicine are two incommensurable medical systems, in using what kind of outcome parameters to assess the therapeutic effect exists great differences. Patients' opinions to the outcome endpoints and preferences should be considered as an important reference. The outcome parameters (such as pain, survival time, etc.) that patient considers relevant and important should be the

indicators recognized by both traditional Chinese medicine and Western medicine. The same disease treated with different therapeutic method and the individual treatment is traditional treatment methods, the efficacy is the joint effect of both doctor's skills and therapeutic effect. How to interpret and promote individualized treatment program of clinical trial results, will be a real problem.

Crossover design unsuitable to traditional Chinese medicine clinical trial

The characteristics of Chinese medicine are slow onset of efficacy, multi- target and complex metabolic process. It is difficult to accurately define the length of wash-out period. Although the onset time of efficacy of Chinese medicine is slow, the lag effects are more obvious, that is, after stopping drug treatment, the drug effects extend a period of time. These features are not suitable for herbal medicine designed as cross-over clinical trial. We had completed two crossover designed clinical trials (benign prostatic hypertrophy and osteoporosis), the results were quite confused after crossover. We expected it might be related to the residual effects of herbal medication.

The importance of FOLLOW-UP for herbal medicine trial

Another special feature of clinical trial for Chinese herbal medicine is the placebo effects are obvious. Sometimes it was quite difficult to distinguish the real drug effect and placebo effect during study period. However, once the drug administration stopped, the placebo effects usually disappear. Because of the residual effects, the real drug effects usually last for a period of time after drug administration stopped. At this period the difference between the placebo and traditional Chinese medicine will be observed. From the point of view of safety, it is also necessary to observe if any side

effect occurred after administration stopped.

In the Subject recruitment:

The inclusion criteria should be consistent, recognized, and authoritative. International diagnosis standards or national uniform standards should be used in inclusion/exclusion design.

The convincing results should be supported by sufficient sample size to achieve statistical test performance. The sample size is usually estimated according to related parameters including previous studies or other similar studies.

Endpoints for efficacy assessment:

Clinical Endpoint means a characteristic or variable that reflects how a patient feels or functions, or how long a patient survives. *Surrogate Endpoint* means a biomarker intended to substitute for a clinical endpoint. A clinical investigator uses epidemiologic, therapeutic, pathophysiologic, or other scientific evidence to select a surrogate endpoint that is expected to predict clinical benefit, harm, or lack of benefit or harm (28).

The appropriate endpoint measure is critical for the efficacy evaluation. Using the wrong endpoint measure could distort the results of a clinical trial. If the endpoint measure fails to capture the intended changes in health or is not sensitive enough to determine clinically meaningful differences, important information about the efficacy of an intervention could be missed (29). Therefore, careful consideration in choosing appropriate endpoint measures is a prerequisite for obtaining valid, clinically useful evidence from TCM clinical trial.

The efficacy assessment of Traditional Chinese medicine is usually based on the

improvement of "*Zheng*". This approach is characterized by focusing on the improvement of physical function of patients as a whole, patient's discomfort, and doctors' personal experience. Limited to macroscopic features of Chinese medicine theory and the constraints of scientific and technological level at that time, traditional Chinese medicine was more concerned on outward signs to determine efficacy, so it might be subjective. It was too vague in the description of parameters, so that it is difficult to form unified objective evaluation criteria to adapt to the needs of current development of medicine. In view of the limitations of traditional methods and the lack of scientific, objective and quantifiable evaluation methods, using internationally accepted standards for efficacy evaluation is our main consideration in designing the protocol. A surrogate outcome should have a strong, independent and consistent association with clinical outcome.

An endpoint is defined as a variable intended for comparison between groups in order to assess the efficacy or harm of an intervention. Endpoints are also commonly referred to as outcomes or variables.

Clinical trials are often constructed with surrogate endpoints for practical or cost considerations. Selecting the most appropriate endpoints in an RCT of TCM remains controversial.

In the selection of parameters, the following points should be fully considered:

(1) Specific endpoints: Specificity is an important consideration as it supports the significance of the findings. The selected endpoints and clinical outcome should have essential relationship or these parameters are able to accurately reflect the performance of the clinical outcome.

(2) Objective endpoints: objective endpoints are often considered more reliable than subjective endpoints. Objective indicators such as laboratory measures are preferred and used for easy inspection and statistical analysis. However, some subjective

endpoints such as pain levels, function, overall health, and how patients feel during or at the end of treatment are valuable. Even in Western medicine, subjective endpoints are commonly used as primary outcomes because they can give a more comprehensive picture of the effectiveness and safety of interventions, especially for functional or psychological disorders.

(3) Sensitive and accurate endpoints: The sensitive indicators that response to the effects of study factors should be chosen, at the same time the indicators should be better accurate.

(4) Proper number of indicators should be considered based on the study purpose, which does not mean the more indicators the better

In order to produce an accurate report of RCTs of TCM, endpoint assessment must be clearly and precisely presented. The primary and secondary outcomes based on the purpose and hypothesis of the trial should be identified. The primary and secondary outcomes should be clearly defined.

In the selection of evaluation parameters, in addition to the principle of objectivity, accuracy and specificity, the following factors need to be considered:

(1) Physical and Chemical Indicators: Adopting the physical and chemical testing indicators that are internationally recognized and operable, relative maturity, which should be reasonable in interpretation of the effectiveness.

(2) Quality of Life (QOL) contains the multi-dimensional concept of bio-medical and social, psychological, spiritual and other factors, which can fully reflect the health status of the human body. Quality of life assessment model is introduced to clinical studies and scientific researches to evaluate the efficacy of Chinese

medicine for refractory diseases. This indicator can show the advantages of Chinese medicine by enhancing the scientific evaluation, the results are easy to be recognized by the mainstream medicine.

(3) Endpoint: Because the refractory disease cannot be cured within a short period, and required a long-term treatment to observe the efficacy and outcomes. Concern of the incidence of major clinical events is also an important objective of clinical treatment. Application of the end-point evaluation in the clinical treatment will improve the level of clinical evaluation of Traditional Chinese Medicine.

Although both Chinese and Western Medicine are aiming at eliminating the diseases of human body, Chinese medicine is characterized by treatment based on syndrome differentiation(辨證論治), which was highlighted with "treating same disease with different methods "(異病同治) and the "treating different diseases with same method"(同病異治), which were more flexible in the individualized treatment compared with Western medicine. The method and parameter selection of efficacy evaluation standards in Western medicine is established under the guidance of theoretical system of Western medicine through large, sample size and homogeneous studies of the clinical trials, focusing on the objective physical and chemical indicators of disease improvement and recovery. In recent years, Western medicine adopts the quality of life as an important indicator for the evaluation of improvement. More important, Chinese medicine and Western medicine have their own different theoretical system, simple copying of the evaluation method of Western medicine to measure the efficacy of traditional Chinese medicine is likely to be acting blindly. However, this method is still used in clinical practice and research in traditional Chinese medicine. The exploration of new scientific and objective efficacy evaluation method that is suitable for TCM research has become the consensus of the majority of

scholars of Chinese medicine.

The clinical research and design of Chinese medicine on the one hand should emphasize its own characteristics and advantages, on the other hand should adopt modern scientific techniques and methods to improve scientific research and design, and to increase the standardization and scientific quality of management for further elevation of the level of clinical research and for providence of high-quality efficacy induce of Chinese medicine.

Specificity of quality control

Chinese medicine has characteristics of complicated composition, multiple active ingredients, and pharmaceutical and toxic ingredients unclear. With the large-scale application of modern analytical techniques, traditional Chinese medicine has got a great leap forward in quality control. The development track is from the traditional identification by the senses to by equipment, appearance-based (macro) method, and then to the molecular (micro) as material-based methods. At present most of the quality control testing methods of traditional Chinese medicine mainly use the physical and chemical analysis methods. In recent years, with the improvement of fingerprinting method for traditional Chinese medicine, the quality control has developed from depending on a single component analysis to the direction of integrated analysis combining the comprehensive analysis with single component analysis.

Although the quality control of traditional Chinese medicine has developed rapidly, there are still some common problems. For example in quality control, an important indicator should be the functional and active ingredients which can reflect the drug safety and effectiveness. However, in the current quality standards for Chinese

Medicines studies, the inappropriate choice of indicators is a common problem. The usually used, high content and easily controlled ingredients, may not be the active ingredients, while the ingredients that are active but low content can not guarantee the correlation between the clinical efficacy and the effective dose. It is not ideal to use it to control and evaluate the quality of traditional Chinese medicine. Use of biological assay to evaluate the quality of Chinese medicine may be better than the physical and chemical assay in evaluation of its biological activity, it is effective on the overall efficacy control.

One of the difficult points in the study of natural medicines is the specific relationship between the complex and diverse chemical composition and the biological activity. Therefore, the only way to resolve the material basis of the pharmacological efficacy of traditional Chinese medicine is to adopt modern technology. The efficacy results should correspond to the chemical composition marked by characteristic peaks of fingerprinting of traditional Chinese medicine. From "micro-analysis" of single component to "macro-analysis" of group composition, and to identify scientific nature between them, the limitations of physical and chemical analysis methods in the current quality control of traditional Chinese medicine and its preparations could be overcome and then the efficacy and consistency can be ensured. It is beneficial to improve the quality control standards and eventually establish a complete quality evaluation system of traditional Chinese medicine and its preparations.

The efficacy of TCM validated with the present Clinical Evaluation methods, gives mostly negative conclusions. Many results of the evaluation given by United States NCCAM-funded clinical trials of Chinese medicine were also negative.

10.7Non-randomized trial in Traditional Chinese Medicine research

The value of observational compared to controlled trial data is a challenging area for Chinese Herbal Medicine research where the importance of observational and outcome research is often espoused. Two therapies may have very different specific and non-specific effects. Depending on the proportion of these effects, a RCT may favor one therapy while the greatest benefit is actually derived from the other (30). Should large observational studies be believed over small RCTs due to the instability of small study results?

The conventional evidence hierarchy would choose RCT data. The evidence house gives each design its due for their respective purposes. Clearly, all domains of research in the evidence house have important places in science. A major challenge to TCM is accommodating the evolving nature of biomedical research by clearly aligning research goals with appropriate methods and not trying to bend the evolution of TCM to predefined methodological conceptions.

10.8 Dose determination of clinical trial for Chinese Herbal Medicine

Dose of Chinese medicine has always been the non-transmitted secret. A well-known fact is that the dose that different schools used sometimes differs in several times, however the efficacy may not show significant difference. In clinical practice we can see that repeatedly prescribing certain herb, the dose can vary up to 25%. These examples make us think that the effective dose of traditional Chinese medicine has a large range of variation and it does not have to be very accurate. Nevertheless, as a drug, traditional Chinese medicine for most people, there should be an optimal dosage.

The variability and complexity of natural compounds make it extremely difficult in most cases to establish a definition of 'equivalence' for botanical drugs and to prove that two 'similar' products are pharmacologically identical or therapeutically interchangeable. Unlike highly purified drugs, the active compounds in botanical drugs are often not identified, and many unknown compounds in the natural mixture could be potentially active.

Because of its complicated chemical composition and lack of concrete evidence of its biological activity, herbal medicine is still not widely accepted by the Western medical community (31).

Dose-response of Chinese medicine, the essence can be understood as the amount of a drug used in the body to cause definite effects, which is also known as effective dose. Dose is expected as efficacy criterion, and efficacy is used to verify the success of a treatment. Different dose implies different effectiveness. The optimal dose selection is usually accompanied by flexibility, difficulty, and thus often arbitrariness and uncertainty. The optimal dose should be having the maximal efficacy with minimal adverse reactions.

Efficacy and dose should be proportional. Within a certain range, with the dose increased the drug effect is also correspondingly increased. The relationship between dose and effect is called the dose-effect relationship (32).

Famous ancient Chinese medicine practitioners were in pursuit of a goal which was using fewer herbs and lower doses to reach maximal effects.

Because of its own characteristics, the dosage of traditional Chinese medicine is inaccurate. One of the reasons is that Chinese medicine itself presents many uncertain

factors. Traditional Chinese medicines are derived from many plants and animals, their quality is affected by various natural factors, many of the factors can not be controlled, so that it is vary difficult to ensure the quality consistency.

The dose-effect relationship of Chinese medicine has the common property of drugs, but also has its distinctive characteristics, such as herbal drug application based on the holistic concept, the dose-effect relationship that formed based on the compatibility of the passion harmony.

The complexity of the dose-effect relationship of traditional Chinese medicine is further reflected in the inter-relationship among the herbs, the passion harmony of compatibility and the relative position of *Jun, Chen, Zhuo, and Shi*, in a formula.

Unlike Western medicine, Chinese medicine has no strict and effective dose, maximum tolerated dose, and therapeutic index. The drug dosage impacts on the effectiveness of herbal formulation, in addition to a total dose of herbal drug prescription, there are also the proportion of herbs, the main medicinal herb dose. Moreover, efficacy of herbal formula is more complex. In view of the herbal drug formula prescription is the main form of clinical application, therefore, the study of dose-effect relationship should focus on the exploration of the dose-effect relationship.

It has been suggested that the biological activity of herbal medicine results from the combination of different active components. When the amount and proportion of active components changed, the biological activity changed accordingly. It has been demonstrated that there exists certain relationship between the biological activity and chemical com position of herbal medicine, and such a relationship is called composition–activity relationship (33, 34, 35).

From the perspective of modern medicine to study the dose - effect relationship of Chinese medicine, the first step is to determine the efficacy parameters. In the selection of efficacy parameter, there are obvious differences between Traditional Chinese Medicine and Western medicine (see Chapter 7). Western medicine emphasizes on objective parameters, while Chinese medicine focuses on subjective feelings. Using the efficacy parameters of Western medicine to research the dose - effect relationship of traditional Chinese medicine is a feasible approach.

Experimental studies, such as random controlled trials (RCT), often provide the most trust-worthy methods for establishing causal relationships from data, in which one or more variable is manipulated (typically randomly) to measure its effect on other variables.

For example, in the clinical trial of exploring the optimal DBT dose, we chose hot flushes, sweating and night sweats as primary efficacy parameters that are commonly used in treatment of postmenopausal women with Western medicine.

Essentially, the relationship between active ingredients of herbal medicine and their biological activities is varied and unclear; the therapeutic effect is expected to change accordingly.

10.9 Quality of Life and TCM efficacy evaluation

World Health Organization (WHO) proposed new concepts of health that not only include physical health but also social life, mental health, and psychological health. In 1985, Quality of Life (QoL), recommended by Food and Drug Administration (FDA), was one of the primary efficacy parameters as a basis for approval of anticancer drugs. The quality of life introduced to the clinical evaluation of Chinese medicine for the

treatment of the diseases is significant in reflecting the characteristics and advantages of Chinese medicine, and promoting the development and modernization of Chinese medicine.

With thousands of years' accumulation of clinical experience, traditional Chinese medicine has particular advantage in improving clinical symptoms, reducing pain and discomfort of patients. However, there is uncertainty in the description of efficacy in TCM clinical evaluation, so it is difficult to objectively and quantitatively evaluate the effects of Chinese medicine. To use scientific methods and techniques to elucidate the efficacy of Chinese medicine is the critical point, otherwise, modernization of Chinese medicine would be seriously affected and the industrialization of Chinese medicine constrained.

It is difficult to objectively and thoroughly evaluate clinical efficacy of the entire characteristics of TCM through a single surrogate, therefore , the outcome selection through the combination of several important symptoms or syndromes, the subjective feeling of patients and their satisfaction of the treatment and also the quality of life can create an objective evaluation for the efficacy of TCM intervention.

The complexity of Chinese herbal medicines and their potential synergistic effect requires innovative evaluative approaches.

The assessment of the health related quality of life and the patient reported outcomes, which are uniformly soft indicators and can be evaluated with the scales of instruments in Western medicine, are identical with the inquiry of traditional Chinese medicine (TCM). The assessing method for the soft indicator in the health related quality of life and the patient reported outcomes was gradually accepted by TCM practitioners and applied in evaluating the curative effect of TCM.

With the changes from bio-medical model to social - biological - psychological medicine pattern in Modern medicine, the parameters of clinical evaluation have significant changes, apart from concerning the physical parameters; patient reported outcomes (PRO) are also concerned. In this point it is quite similar to Traditional Chinese Medicine.

Under the guidance of the bio-medical model of conventional Western medicine, the evaluation of clinical efficacy emphasized on cure rate, improvement rate, morbidity, disability, mortality, and laboratory examinations, laboratory tests and other indicators. Clinically, if the physical and chemical inspection report is normal, the disease is considered cured although the patient feels discomfort or pain, because of lack of laboratory testing support, a diagnosis cannot be made.

Modern medical model changes from the biomedical model to a bio-psychosocial medical model, and disease spectrum also changes from the previous infectious disease to the chronic diseases, which gradually occupy the dominant position of Clinical medicine. Clinically, the outcome of a disease can be divided into three categories: clinical cure, uncured but controlled, and uncured and uncontrolled. After drug treatment most acute illness can be cured, such as acute gastroenteritis, pneumonia, peptic ulcer, etc. The uncured but controlled diseases are mostly chronic, long-term or need lifelong medication diseases, such as hypertension, coronary heart disease, diabetes, chronic obstructive pulmonary disease, chronic kidney disease, autoimmune diseases etc. The uncured and uncontrolled diseases include cancer, motor neurone disease, the aim of treatment is to relieve pain and improve quality of life. It can be seen that the incidence, cure rate and improvement rate of conventional Western medicine cannot fully meet the needs of modern clinical medicine.

The characteristic of traditional Chinese medicine theory is the emphasis of holistic

approach. In treatment of diseases, TCM usually uses herbal formula with a variety of components of Chinese herbal medicine, through a multi-channel, multi-link, multi-target overall level to treat diseases, and from an overall perspective to manage the formation and development of diseases. The characteristics and advantages of TCM treatment are reflected in the macro-level and multi-target management.

The theory and content of Chinese medicine, and the recognition of modern medicine in health is achieving the same understanding. The viewpoint of the overall medical health in Chinese medicine lies in thinking highly of coordination, the multidimensional nature of health factors, and the subjective experience. Its purpose is to regulate the internal and external environment of the human body guided by the holistic concept, to strengthen the patient's condition with the final aim of improving the quality of life. Numerous studies have shown that Chinese medicine plays an important role in improving the quality of life, therefore, quality of life as a new evaluation parameter, was introduced in the efficacy evaluation of Chinese medicine research.

The content and characteristics of quality of life, and Chinese medicine in the medical view of health, has much in common. It means that the quality of life is the evaluation of health from the angle of macro level and holistic approach. This is very similar to the holistic concept of Traditional Chinese Medicine. Therefore, it laid the foundation of the application of quality of life as an important parameter in efficacy evaluation. Quality of life applied in clinical evaluation of Chinese medicine, not only embodies the view of health of Chinese medicine, but also help to highlight the advantages of Chinese medicine as a holistic modulation to fully reflect the effect of Chinese medicine. Quality of life is a common standard in evaluation of Chinese medicine and Western medicine, it reflects the integrated effect, and therefore it is more applicable to treatment of multi-target system such as Chinese medicine. The currently available

QOL instruments may not be sensitive enough to detect the health changes that are regarded as important in CM.

TCM is a holistic approach, and emphasizes the importance of keeping all the structures functioning harmoniously. Modern medicine has a definite index during the intervention of diseases, such as lab index, pathological index, or examination index, while TCM focuses on the improvement of holistic function instead of some index. If the TCM research is conducted only on improved lab index, undoubtedly, no conclusion can be drawn in favor of TCM.

Quality of life (QOL) is a measurement of a person's subjective self-satisfaction, or a subjective evaluation of his/her own living state. If a patient feels pain or discomfort, his QOL decreases, and the QOL will improve when the symptom disappears. TCM has some special advantages in improving symptoms. The symptoms bother the patient, and reduce the self-satisfaction degree. The improvement of distress can significantly enhance the self-satisfaction degree of the patient. Most of the self-satisfaction evaluation toward the symptoms belongs to QOL. In some neurological proceeding diseases like motor neuron diseases (MND) and multiple sclerosis, if breakthroughs cannot be made in the improvement of end points, it is feasible that QOL is regarded as the main evaluation index, and TCM has apparent advantages in these indexes. It is also valuable for the patients.

10.10 Clinical efficacy evaluation methods of TCM

In the long-term clinical practice, Traditional Chinese Medicine has established a systematic and theoretical system, and a unique treatment method. In thousands of years of medical practice, ancient Chinese physicians make a diagnosis mainly

according to the patient's subjective symptoms and tongue, pulse and other indicators as the basis. To determine whether a disease was cured greatly depended on personal experiences (36). Ancient Traditional Chinese medicine documents recorded treatment course in the form of case records and just focusing on the improvement and disappearance of symptoms, and recovery and used it as standards of clinical cure. At present the reports of clinical efficacy of Chinese medicine mostly only stay in the case reports and clinical efficacy summaries (37).

TCM in the treatment of disease emphasizes the *Treatment Depending on Symptom Differentiation* and believes the human body as a whole system undergoes modulation through multi-level, multi-link, multi-target activities to produce effectiveness. Chinese medicine emphasized the clinical efficacy but ignored the methodology of efficacy evaluation. There was little discourse of efficacy evaluation methods in ancient Traditional Chinese Medicine documents. Current clinical evaluation methods of Chinese medicine are consciously or unconsciously copying the evaluation methods and standards of Western medicine, there are many shortcomings in such application.

Scientific and objective clinical multi-dimensional outcome measures of Chinese medicine system should include: 1) accepted conventional efficacy evaluation parameters for the "disease"; 2) the evaluation indicators in composition of *Zheng* changes; 3) assessment of the quality of life including activities of daily living (ADL), the quality of life (QOL) (38).

Based on the characteristics of "holistic approach" of Chinese medicine and in accordance with conventional standards of efficacy evaluation, a multi-dimensional therapeutic effect assessment system including the quality of life can be established, it can provide the evidence showing how Chinese medicine impacts on the quality of

life during disease and sub-health state, and reflecting the real efficacy of Chinese medicine in the prevention & treatment of disease.

Currently we can learn from internationally recognized quality of life scale for the general health evaluation of the population, to establish suitable quality of life for efficacy evaluation of Chinese medicine. While considering in line with international standards, efficacy evaluation system, based on the advantages of Chinese medicine, will help to assess the efficacy of Chinese medicine easy for international acceptance (39, 40).

The clinical efficacy of Chinese medicine has not yet been recognized internationally. The reasons of course are very complicated, in addition to people's cultural background, ways of thinking and clinical research, and the difficulties caused by the characteristics of TCM self in medical research, it is largely due to not giving full attention in the application of the well-designed research methodology. There are many methodological problems affecting the authenticity of the research findings. The use of modern scientific methodology for clinical research of Traditional Chinese Medicine is the age requirements for the development of Chinese medicine (41, 42).

Evidence-based medicine is a new discipline that has a strict grading and evaluation system, efficacy endpoints and the quality of life. It emphasizes clinical efficacy, safety, health economics, ethics and other aspects of comprehensive evaluation of clinical efficacy. It has been widely accepted and recognized by modern medicine. There are many things in common in clinical evaluation method of evidence-based medicine and the holistic approach of Chinese medicine. Therefore, we can draw the principles, methods and results of evidence-based medicine and exert the advantages and characteristics of Chinese medicine concerned on the endpoint and quality of life, to establish an evaluation system that is widely recognized by the mainstream medicine, and that can reflect the characteristics of the evaluation of TCM treatment

methods or systems. The choice of outcome indicators of the Chinese Medicine Clinical Evaluation System should not only base on a simple biomedical model, but also on the overall level. Basing on the foundation of conventional Western medicine of "disease" evaluation standard, we should establish a comprehensive clinical efficacy evaluation system and standard for the evaluation of diseases, syndromes, the quality of life assessment, health economics evaluation, ethics evaluation, and complications, to provide clinical efficacy methods for major diseases, difficult cases, and sub-health status evaluation. So that objective, scientific, and systematic evaluation on TCM clinical efficacy can be made, and the modernization and internationalization of TCM can be improved.

Drawing on the methodology of evidence-based medicine for TCM clinical efficacy evaluation

Evidence-based medicine (EBM) was emerged and developed last century, which was formed from the theories and related knowledge learned and reviewed in clinical research experience and lessons. It is methodology through systematically searching, evaluating and using exist evidence to guide clinical decision-making process. There is a strict classification and evaluation system for clinical research evidence. In effect evaluation, it is focusing on endpoints and quality of life, and emphasizing clinical efficacy, safety, health economics, ethics as integrate clinical evaluation. In the medical profession it has been recognized as a valuable scientific method in clinical decision-making. Traditional Chinese medicine, adopting the principle of evidence-based medicine for clinical evaluation, will allow elevation of direct observation and experience accumulation to a more complete and rigorous level of scientific research, it will benefit the promotion and innovation of Chinese medicine through providing a precise and scientific evidence (43).

Chapter 11

Conclusion

- The research methodology of Traditional Chinese medicine needs to be improved.
- Although the present methods for modern medical research may not be the best for Chinese medicine, but so far it is still the adequate method and cannot be abandoned. The systems biology bridges the integration between Chinese medicine and Western medicine.
- Evidence-based study and efficacy-driven approach, and three-pronged (Clinical trial leading, Biological tests, Quality control) of the research strategy is the best starting point for a comprehensive study of Chinese medicine.
- The purpose of efficacy-driven approach is to obtain the reliable and convincing efficacy evidences of Chinese herbal medicine for herbal drug development.
- Researches for Chinese medicine should focus on the diseases or conditions that Western medicine can not effectively resolve, taking these as the basis research topics and objectives to supplement the insufficiency or as an adjuvant treatment for modern medicine.
- Clinical efficacy evaluation should be based on evidence-based study, by means of double-blind placebo-controlled clinical study to obtain convincing evidence. This is the first choice for Western medicine research, however when it is applied to Chinese medicine, it should be adjusted as follows:
  1) Placebo-controlled clinical study is not the only way for Chinese medicine research. Based on the characteristics of TCM clinical research, the non-randomized controlled study design also can be used.

2) Selection of efficacy endpoints should be mainly based on the objective parameters recognized by mainstream medicine, which will be of benefit to enter the international pharmaceutical markets. However, the parameters that Chinese medicine concerned could be collected in the scope of Quality of Life for further exploration.

3) The judgment of results should take into account its statistical significance and clinical relevance, with consideration of the content that links with Chinese medicine.

- Ancient recipes with hundreds of years' experiences, need to be amended to suit modern needs, and then marketing internationally through commercialization and industrialization.
- Modernization of Chinese medicine should give up the way of westernized medication and turn to the direction of evidence-based herbal formulation research and development.

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Appendix 1

A Randomized, Double-Blind, Placebo Controlled Study of the Effect of Danggui Buxue Tang (當歸補血湯) on Menopausal Symptoms and Quality of Life in Hong Kong Chinese Women

Project Number: ICM/2001/03/004

Protocol Number: ICM/CTS/004

Version: 3

Protocol Date: March 2002

Sponsor:

Institute of Chinese Medicine

The Chinese University of Hong Kong

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Date: _____

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SYNOPSIS

Name of Study TCM: Danggui Buxue Tang (當歸補血湯)
Title of Study: A Randomized, Double-Blind, Placebo-Controlled Study of the Effect of Danggui Buxue Tang (當歸補血湯) on Menopausal Symptoms and Quality of life in Hong Kong Chinese Women
Study center: Single-center
Objectives: ***Primary*** To evaluate the effects of Danggui Buxue Tang (當歸補血湯) on menopausal symptoms of hot flushes and sweating To evaluate the safety of Danggui Buxue Tang (當歸補血湯) in patients with menopausal symptoms ***Secondary*** To evaluate the effect of Danggui Buxue Tang on quality of life of patients with menopausal symptoms To evaluate the effect of Danggui Buxue Tang on some surrogate markers of cardiovascular disease in patients with menopausal symptoms
Design: A single-center, randomized, double blind, placebo-controlled study.
Study Population: A minimum of 100 patients with menopausal symptoms will be enrolled, 50 subjects per group.
Study Regimen: Subjects will be randomly assigned to receive Danggui Buxue Tang (當歸補血湯) or placebo.
Duration of treatment: 6 months.
Statistical Methods: Data will be processed to give group mean values and standard deviations where appropriate. Mann-Whitney U-test will be used to compare the difference between the two groups. Group differences with an error probability of less than 5% ($p<0.05$) will be considered statistically significant. The statistical analyses will be made with SPSS 10.0 for Windows.

Appendix 1

2. INTRODUCTION

2.1 Background

The menopause implies the permanent cessation of menstrual bleeding. In western medicine, this is associated either with the spontaneous failure of normal ovarian function, or it may also result from surgical removal of the ovaries or as a consequence of chemotherapy or radiotherapy. The most common menopausal symptoms are hot flushes and sweating, which are due to a decline in serum oestrogen concentrations. In Caucasian women, hot flushes and sweating have been reported in 70% and 84% respectively after a surgical menopause and in 60% and 74% following a physiological menopause (1). Low oestrogen concentrations are also associated with a decline in quality of life. In the longer term, the menopause is also a associated with an increase in the risk of cardiovascular disease, and the use of oestrogen has been shown to be effective in primary prevention of heart disease in postmenopausal women.

In western medicine, the usual treatment of the menopause is the use of oestrogen replacement therapy. Oestrogen replacement has been shown to be effective in controlling vasomotor symptoms (2). However, treatment with oestrogen may result in unwanted side effects such as breast soreness and nausea. In addition, the long-term safety of oestrogen treatment has not been established. Long-term use may increase the risk of breast cancer and it also increases the risk of venous thrombosis (3). Another long-term consequence of the menopause is deterioration in quality of life. Although there are relatively few publications in this area, studies on both healthy postmenopausal women as well as those that have an underlying medical disorder have suggested that the menopause adversely affects quality of life and that treatment with hormone replacement therapy provides some improvement (4,5). Oestrogen is therefore a valuable treatment for the menopause, but it is not without side effects. It remains to be seen whether Chinese Medicine as described in this proposal can prove to be an effective, safe and well tolerated treatment for the menopause.

From a Chinese medicine perspective, menopausal symptoms are associated

with a decline in Kidney Yin or Yang or a combination of both. Kidney Yang deficiency may manifest itself as hot flushes, night sweating, depression, and other symptoms. Dang Gui (Radix Angelicae Sinensis) is one Chinese herb that is recommended for the treatment of menopausal symptoms. Dang Gui has been shown to depress tachycardia (6), and it also has a protective effect on arteriosclerosis similar to that of oestrogen. Huang Qi is also used in the treatment of the menopausal symptoms to tonify Qi. One study of the effect of Chinese medicinal herbs on menopausal symptoms has shown no beneficial effect, but neither Dang Gui nor Huang Qi was included in the formulation (7). However, not all effects of Chinese herbs may necessarily be beneficial. Dang Gui has been shown to promote bleeding in women using the anticoagulant warfarin (8). Basic safety of these Chinese herbs will also be assessed in this study. This project will examine the effect of a combination of Dang Gui and Huang Qi on menopausal symptoms and on quality of life in postmenopausal Chinese women. It will also examine the effect on various cardiovascular disease risk markers.

2.2 Rationale

Chinese Herbal Medicines containing Dang Gui and Huang Qi have been used for many years to treat menopausal women. Chinese herbal medicine preparations containing Dang Gui are claimed to be efficacious and free of serious side effects. There are few data on possible adverse effects of treatment with Chinese Herbal Medicine containing Dang Gui as well as Huang Qi.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

Primary

- To examine the effect of Danggui Buxue Tang (當歸補血湯) on menopausal symptoms of hot flushes and sweating
- To evaluate the safety of Danggui Buxue Tang (當歸補血湯) in patients with

menopausal symptoms

Secondary

- To evaluate quality of life of patients with menopausal symptoms treated with Danggui Buxue Tang (當歸補血湯).
- To evaluate the effect of Danggui Buxue Tang (當歸補血湯) on various risk markers for cardiovascular disease.

3.2 Study Endpoints

The primary efficacy endpoint is change in severity and frequency of hot flushes and sweats.

The secondary efficacy endpoints are changes in score for the domains measured in the Menopause Specific Quality of Life Questionnaire and changes in values of various markers of risk for cardiovascular disease.

The primary safety endpoint is tolerability. Tolerability failure is defined as a permanent discontinuation of Danggui Buxue Tang (當歸補血湯) as the result of an adverse event.

4. INVESTIGATIONAL PLAN

4.1 Study Design

This is a single-center, randomized, double blind, placebo-controlled study. Subjects who meet the inclusion/exclusion criteria will be randomly assigned to receive Danggui Buxue Tang (當歸補血湯) or placebo in the 6 months study period.

4.2 Study Population

4.2.1 Inclusion Criteria

Menopausal women of any age will be included in the study. Menopausal symptoms will be quantified using the modified Kupperman's index (9).

A subject will be eligible for inclusion if the following apply:

- Follicle stimulating hormone (FSH), luteinizing hormone (LH),

oestradiol in the menopausal range (FSH>18 IU/L, LH>12.6 IU/L, and E2< 361 pmol/l).

- Patients with amenorrhoea for more than 12 months

4.2.2 Exclusion Criteria

General physical examination and pelvic examination will be performed on all patients. If suspected, endometrial pathology will be investigated either by endometrial sampling or outpatient hysteroscopy before the commencement of treatment.

Subjects with any of the following criteria will be excluded:

- Patients with a history of using any form of hormonal replacement therapy within 8 weeks
- Patients with a history of using Chinese medicine or other therapies which may affect the outcome within 8 weeks
- Patients who in the judgment of the investigator will be unable to comply with protocol requirements.
- Patients with significant** gastrointestinal, renal, hepatic, bronchopulmonary, neurological, cardiovascular, breast or endometrial carcinoma, or allergic diseases;
- Patients with uncontrolled hypertension,
- Patients with undiagnosed vaginal bleeding
- Patients with a history of significant drug hypersensitivity.

** Significant is defined as a disease or condition that required hospitalization within the preceding 2 years.

4.3 Treatment During Study

4.3.1 Study Medication and Dosage

Danggui Buxue Tang (當歸補血湯), a combination of Danggui (*Radix Angelicae Sinensis*)當歸 and Huangqi (*Radix Astragali*) 黃芪 will be manufactured by the Hong Kong Institute of Biotechnology based on GMP (Good Manufacturing Practice) standards.

The study medication will be formulated into uniform dose tablet or capsule

under the supervision of the Clinical Trials Section of Institute Chinese Medicine of the Chinese University of Hong Kong.

The dosage of Danggui Buxue Tang (當歸補血湯) will be 3g (6 capsules / tablets) per day.

The placebo will also be given as 6 capsules or tablets /day. Danggui Buxue Tang (當歸補血湯) will be prepared with the same color and size as the placebo. Patients in both groups will take the same amount of the drug.

4.3.2 Study Treatment Assignment

The patients will be assigned to receive their allocated treatment according to a computerized generated randomization table prepared at the Center for Clinical Trials & Epidemiological Research, the Chinese University of Hong Kong before the start of the study.

4.3.3 Dosage & Administration

Danggui Buxue Tang (當歸補血湯) (3g daily) and placebo used in the clinical trial will be given orally.

4.3.4 Concurrent Medications and Non-Drug Therapies

Patients may receive medication for concomitant diseases provided that it does not interfere with the outcome measures. Any use of concomitant medication will be reported in the appropriate section of the case report form and, if possible, will be kept at the same dosage level for the duration of the study.

5. STUDY MEDICATION MANAGEMENT

5.1 Study Medication Packaging and Labeling

Danggui Buxue Tang (當歸補血湯) will be packaged and labeled according to the standard operating procedures of the Hong Kong Institute of Biotechnology Ltd., in order to protect the product from deteriorating during transportation and storage.

Description of packaging:

The packaging of Danggui Buxue Tang (當歸補血湯) and placebo will be identical.

Contents of the labels:

The label for each study drug, either Danggui Buxue Tang(當歸補血湯) or placebo, will include the name of the institute, study number, subject initial, and number of study visit.

5.2 Study Medication Handling

The study medication will be stored at room temperature.

The sponsor will be responsible for the safety and storage of the study medications.

5.3 Study Medication Accountability Procedures

Responsibility for study medication accountability at the trial site rests with the investigator. The investigator may assign some of the investigator's duties for the study medication accountability at the trial site to an appropriate person. The investigator must ensure that the study medication is used only in accordance with the protocol.

The investigator will maintain records, which document the delivery of the study medication to the subjects, the quantity of the product used by each subject and the quantity returned to the sponsor. The sponsor will maintain records that document adequately that the subjects were provided the doses specified by the protocol and reconcile all study medication received from the sponsor.

6. PROCEDURES AND METHODS

6.1 GCP Compliance and Monitoring

The study will be performed in accordance with Good Clinical Practice (GCP), the Principles of the Declaration of Helsinki and applicable local customs and laws.

6.2 Documentation

The investigator will maintain a comprehensive patient record during patient visits as this constitutes source data. Relevant data will then be transcribed into the Case Record Form (CRF).

CRFs will be completed legibly for each patient enrolled in the study and signed by the investigator. This will be done as soon as possible after completion of a study visit.

6.3 Informed Consent and Patient Information

Informed consent will be obtained according to the local laws and the Good Clinical Practices Guidelines, prior to the inclusion in the clinical study and assignment of the patient study number. Patients will have a discussion about the study and voluntarily sign and date an informed consent form.

Patients will also be given information about the nature, significance and scope of the study, tests to be performed and potential risks. Patients will also be informed about their right to revoke their consent at any time without an obligation to explain the reason and without prejudice to their further treatment.

6.4 Assessment Periods

Only patients who have given their informed consent to participate in the study will be included in the screening. Patients should adhere to the visit schedule outlined below and return to the clinic on the days indicated. The Baseline Visit should always be used as the reference visit for identifying days of study and visit dates. The window limit on a visit is 7 days prior to and 7 days after the visit.

Screening Period/Visit 0 (Day -30 to -1)

Prescreened patients who appear likely to meet the eligibility criteria will be asked to participate in the study and brought into the clinic for a Screening Visit. Once a patient meets all of the eligibility criteria (i.e. after laboratory results have been obtained), a Baseline Visit will be scheduled. A randomization number will be assigned to each patient, after which that

number **cannot** be used for any other patient.

Baseline / Visit 1

The Baseline at visit 1 is the last day of the Screening period and will occur within 30 days of the Screening Visit.

Treatment period (day 1 to day 182)

The period will last for 6 months (i.e. day 1 to day 182). Patients will receive Danggui Buxue Tang (當歸補血湯) or placebo and will visit their physician on Day 91/Visit 2, and Day 182/Visit 3.

Appendix 1

6.5 Schedule of Assessments

Period	Screening	Baseline	Treatment	
Visit	**0**	**1**	**2**	**3**
Day	-30 to -1	0	91	182
Medical History	X	X	X	X
Menopausal Symptoms	X	X	X	X
Vital Signs	X	X	X	X
Physical Examination	X			
Review of Incl./Excl. Criteria Study	X			
Informed Consent[a]	X			
Urinalysis	X		X	X
Concomitant Medications	X	X	X	X
Randomization		X		
Hormone assay	X		X	X
Hematology	X		X	X
Biochemistry	X		X	X
Pelvic Ultrasound Endometrial Sampling/ Hysteroscopy	X			
Vaginal Maturation	X			X
Body Mass Index (kg/m^2)		X	X	X
Cardiovascular markers		X	X	X
MENQOL[c]		X	X	X
Arterial Reactivity		X	X	X
Dispense Study Medication		X	X	
Medication Accountability			X	X
Record Adverse Event			X	X

6.6 Assessment for Treatment Effects

Screening Period/Visit 0

The following observations and measurements will take place during the Screening Visit:

- Obtain medical history, gynaecological history and demographic data
- Record the severity and frequency of vasomotor symptoms
- Record height, body mass index, and vital signs
- Perform clinical examination
- Review inclusion and exclusion criteria
- Obtain informed consent from the patient
- Collect specimen for urinalysis
- Record concomitant medications
- Advise discontinuation of all medication not permitted in this trial
- Pelvic ultrasound ± biopsy
- Vaginal maturation index
- Obtain samples for hematology, hormone assay and biochemistry

Baseline / Visit 1

Procedure/assessments that must occur during the Baseline Visit include:

- Medical history
- Record the severity and frequency of vasomotor symptoms
- Randomize the patient (i.e. Baseline Visit only).
- Record body mass index and vital signs.
- Record the score of MENQOL
- Cardiovascular markers
- Brachial artery reactivity
- Record concomitant medications
- Dispense medication

Visit 2, Day 91

- Medical history
- Record the severity and frequency of vasomotor symptoms

- Record body mass index and vital signs.
- Obtain samples for hematology, hormone assay and biochemistry analysis
- Collect specimen for urinalysis.
- Record the score of MENQOL
- Record adverse events and concomitant medications
- Cardiovascular markers
- Brachial artery reactivity
- Dispense medication
- Medication accountability

Visit 3, Day 182

- Medical history
- Record the severity and frequency of vasomotor symptoms
- Record body mass index and vital signs.
- Obtain samples for hematology, hormone assay and biochemistry analysis
- Collect specimen for urinalysis.
- Record the score of MENQOL
- Record adverse events and concomitant medications.
- Vaginal maturation index
- Cardiovascular markers
- Brachial artery reactivity
- Medication accountability

6.7 Clinical Laboratory Tests

Biochemistry, Hematology, Hormone Assay and Cardiovascular markers

The laboratory assessments will be performed in the Prince of Wales Hospital. The following will be performed:

Appendix 1

Hematology:	Hemoglobin WBC count Platelets
Biochemistry:	Glucose Creatinine Albumin Plasma Urea ALP ALT/GPT ALT Total bilirubin Sodium Potassium
Urinalysis:	Glucose Protein acetone (ketone), pH
Hormone assay	FSH LH E2
Cardiovascular markers	Highly sensitive CRP Homocysteine Lipid Profile

6.8 Patient Medication Diary Card

The Patient Medication Diary Card will be issued to each patient at every visit starting from the Baseline Visit. Patients will be required to record the date of the use of the study medication during the study. The data collected from the patient's diaries will be collected at each visit and the compliance will be checked by counting the remaining tablets/capsules.

6.9 Premature Discontinuation

6.9.1 Premature Withdrawal of Patients from the Study

Subjects must be withdrawn from the study if the subject withdraws consent. The withdrawal of consent may take place at any time; no justification for such a decision is required. Subjects will be encouraged to complete the study; however, they may voluntarily withdraw at any time. Nevertheless, if the subject withdraws from the study, a Premature Termination Visit should be made within 3 days after the last dose of the study medication.

A subject may discontinue taking the study medication for the following medical or administrative reasons, but will be followed per protocol until the end of the study:

- Adverse event(s) deemed sufficiently serious to require discontinuation of study medication
- At the discretion of the investigator
- Violation of eligibility criteria
- Deviation from the treatment plan specified in the protocol
- Progression of disease which, in the opinion of the principal investigator, should preclude further study
- Clinically significant intercurrent therapy which is thought to interfere with the outcome of this study
- Patient non-compliant with the study protocol.
- Patient lost to follow-up.

6.9.2 Premature Termination of Study/Closure of Study Site

Conditions that may warrant termination of the study include, but are not limited to:

- The discovery of an unexpected, serious, or unacceptable risk to the patients enrolled in the study.
- The decision to suspend or discontinue testing, evaluation, or development of the test agent.

7. STATISTICAL ANALYSIS

Data will be processed to give group mean values and standard deviations where appropriate.

Thereafter each continuous variable will be tested for homogeneity of variance with Bartlett's test. If the variance is homogeneous, analysis of variance will be carried out for the variable. If any significant difference is detected, possible intergroup differences will be assessed with Dunnett's test. If the variance is heterogeneous, each variable will be tested for normality by the Shapiro-Wilk method. In case of normal distribution, t-test will be used to compare the difference between the two groups of treatment. Otherwise the non-parametric test of Mann-Whitney U-test will be used.

Group differences with an error probability of less than 5% ($p<0.05$) will be considered statistically significant.

The statistical analyses will be made with SPSS 10.0 for windows.

Determination of sample size

Assuming similar efficacy to that of oestradiol in eliminating hot flushes (expect reduction in prevalence from 0.67 to 0.32), with alpha=0.05 and power 0.90 need 41 patients / group. Therefore, to allow for approximately 20% dropouts, 100 women will be recruited.

8. SAFETY MANAGEMENT

8.1 Definition of Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product. It does not necessarily have a causal relationship with this treatment.

An adverse event (AE) can therefore be an unfavorable and unintended sign (that could include a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical product, whether or not considered related to the medical product.

An adverse event **does** include:

- An exacerbation of a pre-existing illness
- An increase in frequency or intensity of a pre-existing episodic event or condition
- A condition detected or diagnosed while on a study, which may have been present prior to the start of the study
- Continuous persistent disease/symptoms present at baseline that worsen following the start of the study

An adverse event **does not** include:

- Medical or surgical procedures (e.g. surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an AE
- Pre-existing disease or conditions present or detected at the start of the study that do not worsen
- Situations where an untoward medical occurrence has not occurred (e.g. hospitalizations for cosmetic elective surgery, social/convenience admissions)
- The disease being studied or signs/symptoms associated with the disease unless more severe than expected for the patient's condition
- Overdose of study medication or concurrent medication without any signs or symptoms

The intensity of each adverse event will be graded on a three point scale (mild, moderate and severe) and reported in detail as indicated on the Case Report Form.

Mild: Adverse event usually transient in nature and generally not interfering with normal activities.

Moderate: Adverse event, which is sufficiently discomforting to interfere with normal activities.

Severe: Adverse event, which is incapacitating and prevents normal activities.

8.2 Definition of Adverse Reaction

All noxious and unintended responses to a medicinal product related to any

dose will be considered adverse reactions.

8.3 Definition of Serious Adverse Event

In order to standardize investigator evaluation of safety, the use of the following standard assessment for the variable seriousness, labeling, intensity, outcome, and causality is imperative:

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Is fatal (results in death)
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Requires inpatient hospitalization
- Prolongs existing inpatient hospitalization

- **8.4 Adverse Event(s) Reporting**

All adverse events must be fully recorded on the Adverse Event Page of the Case Record Form (CRF). Documentation must be supported by an entry in the patient's medical record.

Laboratory test abnormalities considered by the Investigator to be clinically relevant will be reported on the Adverse Event Page of the CRF.

Signs and symptoms of each adverse event will be described in detail: date of onset, intensity, outcome, date of resolution, action taken with the study function, relationship to the study TCM and the seriousness of the adverse event.

8.5 Serious Adverse Event(s) Reporting

All serious adverse event whether or not believed to be drug-related must be reported to the Clinical Trials Section, Institute of Chinese Medicine (ICM), The Chinese University of Hong Kong by FAX (or telephone) within 24 hours upon the discovery of the event.

The Investigator is responsible to complete the Serious Adverse Event Report Form and forward to the ICM within 24 hours upon the discovery of the event.

The Sponsor will review the information and contact the investigator for

additional information or documentation, if necessary.

8.6 Clinically Significant Abnormalities

Laboratory analysis is frequently performed in the clinical trial. An abnormal laboratory finding is not by itself considered to be an AE or a serious AE (SAE) unless the investigator thinks the abnormal finding is of clinical significance and should be reported in such a manner. The abnormal laboratory finding does not have to be associated with the use of the test agent to be considered clinically significant. Those experiences that require special reporting are described above.

It is the responsibility of the Investigator to review and assess the clinical significance of all abnormal laboratory values as defined by the list of normal values. All abnormal laboratory tests that are judged to be at least possibly treatment related or of uncertain causality must be repeated. For any significant changes noted by the investigator to be clinically significant or those that meet the criteria above, the clinical significance and relationship to the administration of the study treatment will be established. This assessment will be recorded on the CRF. If the changes are clinically significant, the patient will stop taking the study medication immediately. If the abnormal laboratory values do not return to normal within 14 days, the patient should be withdrawn from the study. In this case, the investigator will continue to monitor the patient until the parameter returns to normal or until the investigator determines that follow-up is no longer medically necessary.

8.7 Follow-up of Serious Adverse Event(s)

If required, a follow-up report including all relevant new or reassessed information (e.g. concomitant medication, medical history) obtained on the SAE must be prepared using a second SAE form marked *follow-up.* This report should be faxed to the ICM within the same timeframe specified for reporting SAE.

The Investigator and supporting personnel responsible for patient care will institute any supplementary investigations of serious adverse events based on their clinical judgement of the likely causative factors.

8.8 Local Ethics Committee Notification of Serious Adverse Event(s)

The Investigator is responsible for promptly notifying the local ethics committee of all serious adverse events, including follow-up information.

8.9 Notification of Sponsor of Post-Study Adverse Event(s)

The Investigator will notify the Sponsor of any death or serious adverse event occurring at any time after a subject has signed out of a clinical trial, when such death or adverse event may reasonably be related to the treatment used in the study. Investigators are not obligated to actively seek adverse events in former study participants.

8.10 Breaking the Treatment Code due to AE(s) and SAE(s)

An emergency envelope containing the randomization code will be printed for each subject and must not be broken except for emergency situations. The investigator must inform the Sponsor immediately of any broken code. Any non-blinding of the study medication must be documented and explained by the investigator in the CRF.

9. INVESTIGATOR OBLIGATIONS

9.1 Data Collection

The investigator will maintain the individual patient files separate from the Case Record Form (CRFs). The individual patient files are considered source data and will include: visit dates of the patient, date of informed consent, date of randomization, medical history, physical examination, laboratory results, concomitant medications and treatments, any adverse events encountered and other notes as appropriate. All entries on the CRFs must be backed up by source data.

9.2 Study Records and Source Documents

Source documents may include subject's medical records, hospital charts, clinic charts, the Investigator's study files, as well as the results of diagnostic tests. The Investigator's copy of the CRF serves as the Investigator's record of a subject's study-related data.

9.3 Disclosure

Individual subject medical information obtained as a result of this study is considered confidential, and disclosure to third parties other than those noted below is prohibited

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Appendix 2

A Randomized, Double-Blind, Multiple-Dose Escalation Study of the Effect of Danggui Buxue Tang (DBT 當歸補血湯) on symptomatic postmenopausal Hong Kong Chinese Women
(Continuation of a completed study using the same formula)

Protocol Number: ICM/CTS/05/336

Version: 4

Protocol Date: September 2005

Grant :

UGC-Area of Excellence (AoE) Project :Chinese Medicine Research and Further Development

Collaborator:

Institute of Chinese Medicine (ICM)

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Appendix 2

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Appendix 2

SYNOPSIS

Name of Study TCM: Danggui Buxue Tang (當歸補血湯) (An AoE project)
Title of Study A Randomized, Double-Blind, Multiple-Dose Escalation Study of the Effect of Danggui Buxue Tang (當歸補血湯) on symptomatic postmenopausal Hong Kong Chinese Women (Continuation of a completed study using the same formula)
Study center: Single-center
Objectives: ***Primary*** To examine optimal dose of Danggui Buxue Tang (當歸補血湯) in treatment of menopausal symptoms of hot flushes and sweating To evaluate the safety and tolerability of Danggui Buxue Tang (當歸補血湯) in patients with menopausal symptoms ***Secondary*** To evaluate the effect of Danggui Buxue Tang on quality of life of patients with menopausal symptoms
Design: A single-center, randomized, double blind, multiple-dose escalation study.
Study Population: A minimum of 60 patients with menopausal symptoms will be enrolled, 20 subjects per group.
Study Regimen: Subjects will be randomly assigned to receive different dose of Danggui Buxue Tang (當歸補血湯).
Duration of treatment: 3 months.
Statistical Methods: Data will be processed to give group mean values and standard deviations where appropriate. Mann-Whitney U-test will be used to compare the difference between the two groups. Group differences with an error probability of less than 5% ($p<0.05$) will be considered statistically significant. The statistical analyses will be made with SPSS 10.0 for Windows.

Appendix 2

2. INTRODUCTION

2.1 Background

The menopause implies the permanent cessation of menstrual bleeding. In western medicine, this is associated either with the spontaneous failure of normal ovarian function, or it may also result from surgical removal of the ovaries or as a consequence of chemotherapy or radiotherapy. The most common menopausal symptoms are hot flushes and sweating, which are due to a decline in serum oestrogen concentrations. In Caucasian women, hot flushes and sweating have been reported in 70% and 84% respectively after a surgical menopause and in 60% and 74% following a physiological menopause (1). Low oestrogen concentrations are also associated with a decline in quality of life. In the longer term, the menopause is also associated with an increase in the risk of cardiovascular disease, and the use of oestrogen has been shown to be effective in primary prevention of heart disease in postmenopausal women.

In western medicine, the usual treatment of the menopause is the use of oestrogen replacement therapy. Oestrogen replacement has been shown to be effective in controlling vasomotor symptoms (2). However, treatment with oestrogen may result in unwanted side effects such as breast soreness and nausea. In addition, the long-term safety of oestrogen treatment has not been established. Long-term use may increase the risk of breast cancer and it also increases the risk of venous thrombosis (3). Another long-term consequence of the menopause is deterioration in quality of life. Although there are relatively few publications in this area, studies on both healthy postmenopausal women as well as those that have an underlying medical disorder have suggested that the menopause adversely affects quality of life and that treatment with hormone replacement therapy provides some improvement (4,5).

Oestrogen is therefore a valuable treatment for the menopause, but it is not without side effects. It remains to be seen whether Chinese Medicine as described in this proposal can prove to be an effective, safe and well tolerated treatment for the menopause.

From a Chinese medicine perspective, menopausal symptoms are associated with a decline in Kidney Yin or Yang or a combination of both. Kidney Yang deficiency may manifest itself as hot flushes, night sweating, depression, and other symptoms. Dang Gui (Radix Angelicae Sinensis) is one Chinese herb that is recommended for the treatment of menopausal symptoms. Dang Gui has been shown to depress tachycardia (6), and it also has a protective effect on arteriosclerosis similar to that of oestrogen. Huang Qi is also used in the treatment of the menopausal symptoms to tonify Qi. One study of the effect of Chinese medicinal herbs on menopausal symptoms has shown no beneficial effect, but neither Dang Gui nor Huang Qi was included in the formulation (7). However, not all effects of Chinese herbs may necessarily be beneficial. Dang Gui has been shown to promote bleeding in women using the anticoagulant warfarin (8).

We have now shown a beneficial effect of Danggui Buxue Tang on vasomotor symptoms in postmenopausal Chinese women (Re: CREC Ref. No. CRE-2002.152-T Principal Investigator: Prof. / Dr. Haines Christopher John Protocol Title: A Randomized Double-Blind Placebo-Controlled Study of The Effect Of Danggui Preparation On Menopausal Symptoms And Quality Of Life In Hong Kong Chinese Women). In a similar study population to that which will be used for this proposal, we found that the incidence of vasomotor symptoms was reduced in users of Danggui Buxue Tang compared to those using placebo. However, we could not show a difference in the reduction in severity of symptoms between Danggui Buxue Tang and placebo. We now wish to confirm our initial findings, and we hope to obtain more reliable data by (1) only including women who have never used any type of

treatment for their menopause and (2) by recruiting women who have more severe symptoms of the menopause.

2.2 Rationale

Chinese Herbal Medicines containing Dang Gui and Huang Qi have been used for many years to treat menopausal women. Chinese herbal medicine preparations containing Dang Gui are claimed to be efficacious and free of serious side effects. There are few data on possible adverse effects of treatment with Chinese Herbal Medicine containing Dang Gui as well as Huang Qi.. The basic components of DBT include Radix Angelicae sinensis (one part) and Radix Astragali membranaceus (five parts).

3. STUDY OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

The objective of present study is to investigate the dose response relationship to assess an optimal dose suitable for clinical use.

Primary

- To examine the appropriate dose of Danggui Buxue Tang (當歸補血湯) in the treatment of menopausal symptoms of hot flushes and sweating
- To evaluate the tolerability and safety of Danggui Buxue Tang (當歸補血湯) in patients with menopausal symptoms

Secondary

- To evaluate quality of life of patients with menopausal symptoms treated with Danggui Buxue Tang (當歸補血湯).

3.2 Study Endpoints

The primary efficacy endpoint is change in severity and frequency of hot flushes and sweats.

Appendix 2

The secondary efficacy endpoints are changes in score for the domains measured in the Menopause Specific Quality of Life Questionnaire and changes in values of various markers of risk for cardiovascular disease.

The primary safety endpoint is tolerability. Tolerability failure is defined as a permanent discontinuation of Danggui Buxue Tang (當歸補血湯) as the result of an adverse event.

4. INVESTIGATIONAL PLAN

4.1 Study Design

Our stage 1 randomized double blind placebo controlled clinical trial (Phase II) has demonstrated that DBT has effects in improving vasomotor symptoms (hot flushes, sweating and night sweats) over the placebo group. In this Stage II clinical trial, we are planning to investigate the optimum safe and tolerable dose for clinical application.

For the present stage 2 clinical trial, the principle of phase I clinical trial will be considered and implemented in the design. The trial will be designed as a multiple-dose escalation clinical trial to obtain accurate information on the efficacy and safety when used for menopausal women. Since stage 1 has already confirmed that the traditional dose (normal) is efficacious, the main purpose of the study is to look for an optimal dose for the treatment of menopausal symptoms.

Therefore three arms will be used: one arm will be treated with the normal dose which is the same as stage 1 trial; the second arm will be treated with a dose 100% higher than the normal dose and the third arm with a dose 50% lower than the normal dose. Subjects who meet the inclusion/exclusion criteria will be randomly assigned to receive different doses of Danggui Buxue Tang (當歸補血湯) for 3 months.

Appendix 2

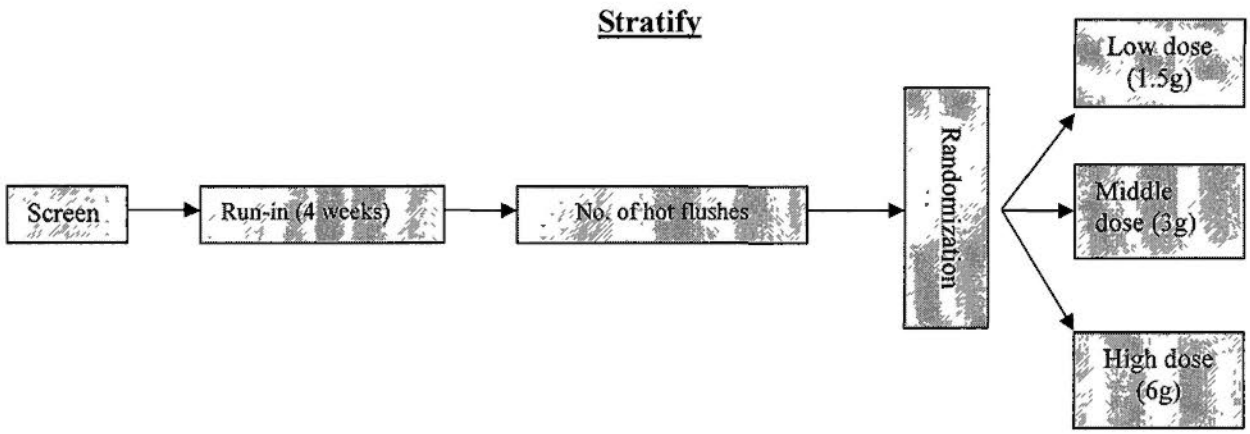
Stratification factors

- Number of hot flushes: 14-21/week vs. >21 / week

Randomization procedures

After the patient has been registered into the study, the values of the stratification factors will be recorded, and the patient will be randomly assigned to one of the following treatment groups:

- Group 1: will take DBT 1.5g daily for 3 months
- Group 2: will take DBT 3g daily for 3 months
- Group 3: will take DBT 6g daily for 3 months



Dosage Determination

Previous Stage I clinical trial with the dose of 3g (6 capsules) daily has demonstrated the effects of DBT in the treatment of vasomotor symptoms (hot flashes and night sweats). The present study will be designed as multi-dose escalation study to find out optimal dose. Three dosage groups will be set:

- High dosage: 6 grams daily
- Middle dosage: 3 grams daily, same as previous clinical trial dosage
- Low dosage: 1.5 grams daily

Appendix 2

In completed Stage I clinical trial. the results indicated that the improvement of vasomotor symptoms usually occurred after 4 or 5 months of DBT treatment. The daily dosage of 3 grams extracted from the mixture of Dang Gui and Huang Qi in the ratios of 1 to 5 (w/w) is equivalent to 8.8g of raw herbs, based on the extraction rate of 34% provided by Hong Kong Institute of Biotechnology (HKIB). This dosage is only one-fourth of traditional dosage according to the record of 《内外傷辨惑論》, in which the formula was consisted of Dang Gui 6grams and Huang Qi 30 grams, total 36 grams for daily dose.

Previous safety studies have demonstrated that both Dang Gui and Huang Qi have very low toxicity. The LD_{50} of Dang Gui in mice is 100g /kg via injection. which is 1000 times higher than human dose. Long-term ingestion at the dosage of 6g/kg showed no abnormality in physical activity, food intake. body weight, urine examination, or hematological examination. This dose is 60 times higher than human dose. Usually. the recommended dosage of Dang Gui for human is 5 to 15 grams. Huang Qi is also safe, doses as high as 100g/kg of raw herb had been given by gavage to rats with no adverse effects. Oral ingestion of Huang Qi decoction (7.5g/kg) cannot be determined in rats. The LD50 in mice for intraperitoneal injection is approximately 40g/kg. The recommended human dose is usually 10 to 15 grams. The maximum dosage of Huang Qi is up to 120 grams.

Above evidence is summarized as follows:

- Old dose is only one quarter of the traditional dosage and was taken for a long time (4 or 5 months)
- The newly recommended high dose (6g/day) is about one-half of the traditional dosage
- The new dose is much lower than safety dosages

Appendix 2

- The dose of individual herbs is still within the recommended human dosage
- The duration of DBT treatment has been reduced to 3 months

4.2 Study Population

4.2.1 Inclusion Criteria

Menopausal women of any age will be included in the study. Menopausal symptoms will be quantified using the modified Greene Climacteric Scale. They will also be assessed as part of the MENQOL quality of life questionnaire.

A subject will be eligible for inclusion if the following apply:

- Follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol in the menopausal range (FSH>18 IU/L, LH>12.6 IU/L, and E2< 361 pmol/l).
- Patients with amenorrhoea for more than 12 months
- Never received treatment for menopausal symptoms
- Never received menopausal hormone therapy
- Reporting a minimum of 14 hot flushes per week at the time of entry into the study

4.2.2 Exclusion Criteria

General physical examination and pelvic examination will be performed on all patients. If suspected, endometrial pathology will be investigated either by endometrial sampling or outpatient hysteroscopy before the commencement of treatment.

Subjects with any of the following criteria will be excluded:

- Patients with a history of using Chinese medicine or other therapies

which may affect the outcome within 8 weeks

- Patients who in the judgment of the investigator will be unable to comply with protocol requirements.
- Patients with significant** gastrointestinal, renal, hepatic, bronchopulmonary, neurological, cardiovascular, breast or endometrial carcinoma, or allergic diseases;
- Patients with uncontrolled hypertension,
- Patients with undiagnosed vaginal bleeding

** Significant is defined as a disease or condition that required hospitalization within the preceding 2 years.

4.3 Treatment During Study

4.3.1 Study Medication and Dosage

Danggui Buxue Tang (當歸補血湯), a combination of Danggui (*Radix Angelicae Sinensis*)當歸 and Huangqi (*Radix Astragali*) 黃芪 will be manufactured based on GMP (Good Manufacturing Practice) standards.

The study medication will be formulated into uniform dose granules under the supervision of the Centre for Clinical Trials on Chinese Medicine (CCTCM), Institute of Chinese Medicine of the Chinese University of Hong Kong.

Three dosages of Danggui Buxue Tang (當歸補血湯) will be set:

- 6g per day
- 3g per day
- 1.5g per day

Different dosage of Danggui Buxue Tang (當歸補血湯) will be prepared with the same color and size. Patients in different dosage groups will take the same amount of the drug.

4.3.2 Study Treatment Assignment

The patients will be assigned to receive their allocated treatment according to a computerized generated randomization table prepared at the Center for Epidemiology and Biostatistics, the Chinese University of Hong Kong before the start of the study.

4.3.3 Dosage & Administration

Different dosage of Danggui Buxue Tang (當歸補血湯) will be given orally.

4.3.4 Concurrent Medications and Non-Drug Therapies

Patients may receive medication for concomitant diseases provided that it does not interfere with the outcome measures. Any use of concomitant medication will be reported in the appropriate section of the case report form and, if possible, will be kept at the same dosage level for the duration of the study.

5. STUDY MEDICATION MANAGEMENT

5.1 Study Medication Packaging and Labeling

Danggui Buxue Tang (當歸補血湯) will be packaged and labeled according to standard operating procedures in order to protect the product from deteriorating during transportation and storage.

Description of packaging:

The packaging of different dosage of Danggui Buxue Tang (當歸補血湯) will be identical.

Contents of the labels:

The label for different dose of Danggui Buxue Tang(當歸補血湯) will include the name of the institute, study number, subject initial, and number of study visit.

5.2 Study Medication Handling

The study medication will be stored at room temperature.

CCTCM will be responsible for the safety and storage of the study medications.

5.3 Study Medication Accountability Procedures

Responsibility for study medication accountability at the trial site rests with the investigator. The investigator may assign some of the investigator's duties for the study medication accountability at the trial site to an appropriate person. The investigator must ensure that the study medication is used only in accordance with the protocol.

The investigator will maintain records, which document the delivery of the study medication to the subjects, the quantity of the product used by each subject and the quantity returned to the sponsor. CCTCM will maintain records that document adequately that the subjects were provided the doses specified by the protocol and reconcile all study medication received from the sponsor.

6. PROCEDURES AND METHODS

6.1 GCP Compliance and Monitoring

The study will be performed in accordance with Good Clinical Practice (GCP), the Principles of the Declaration of Helsinki and applicable local customs and laws.

6.2 Documentation

The investigator will maintain a comprehensive patient record during patient visits as this constitutes source data. Relevant data will then be transcribed into the Case Record Form (CRF).

CRFs will be completed legibly for each patient enrolled in the study and signed by the investigator. This will be done as soon as possible after completion of a study visit.

6.3 Informed Consent and Patient Information

Informed consent will be obtained according to the local laws and the Good Clinical

Practices Guidelines, prior to the inclusion in the clinical study and assignment of the patient study number. Patients will have a discussion about the study and voluntarily sign and date an informed consent form.

Patients will also be given information about the nature, significance and scope of the study, tests to be performed and potential risks. Patients will also be informed about their right to revoke their consent at any time without an obligation to explain the reason and without prejudice to their further treatment.

6.4 Assessment Periods

Only patients who have given their informed consent to participate in the study will be included in the screening. Patients should adhere to the visit schedule outlined below and return to the clinic on the days indicated. The Baseline Visit should always be used as the reference visit for identifying days of study and visit dates. The window limit on a visit is 7 days prior to and 7 days after the visit.

Screening Period/Visit 0 (Day -30 to -1)

Prescreened patients who appear likely to meet the eligibility criteria will be asked to participate in the study and brought into the clinic for a Screening Visit. Once a patient meets all of the eligibility criteria (i.e. after laboratory results have been obtained), a Baseline Visit will be scheduled. A randomization number will be assigned to each patient, after which that number **cannot** be used for any other patient. During screening, all patients must have reported on average 21 or more hot flushes per week.

Baseline / Visit 1

The Baseline at visit 1 is the last day of the Screening period and will occur within 30 days of the Screening Visit.

Treatment period (day 1 to day 91)

Appendix 2

The period will last for 3 months (i.e. day 1 to day 92). Patients will receive Danggui Buxue Tang (當歸補血湯) or placebo and will visit their physician on Day 31/Visit 2, and Day 92/Visit 3.

Appendix 2

6.5 Schedule of Assessments

Period	Screening/ Run-in	Baseline	Treatment		Follow-up
Visit	**0**	**1**	**2**	**3**	**4**
Day	**-30 to -1**	**0**	**31**	**92**	**120**
Medical History	X				
Menopausal Symptoms	X	X	X	X	X
Vital Signs	X	X	X	X	X
Physical Examination	X				
Review of Incl./Excl. Criteria Study	X				
Informed Consent[a]	X				
Urinalysis	X		X	X	X
Concomitant Medications	X	X	X	X	X
Randomization		X			
Hormone assay	X		X	X	X
Hematology	X		X	X	X
Biochemistry	X		X	X	X
Pelvic Ultrasound Endometrial Sampling/ Hysteroscopy	X				
Vaginal Maturation	X			X	X
Body Mass Index (kg/m^2)		X	X	X	X
Greene Scale	X	X	X	X	X
MENQOL[c]	X	X	X	X	X
Dispense Study Medication		X	X		
Medication Accountability			X	X	

Record Adverse Event			X	X	

6.6 Assessment for Treatment Effects

Screening Period/Visit 0

The following observations and measurements will take place during the Screening Visit:

- Obtain medical history, gynaecological history and demographic data
- Record the severity and frequency of vasomotor symptoms
- Record height, body mass index, and vital signs
- Perform clinical examination
- Review inclusion and exclusion criteria
- Obtain informed consent from the patient
- Collect specimen for urinalysis
- Record concomitant medications
- Advise discontinuation of all medication not permitted in this trial
- Pelvic ultrasound
- Obtain samples for hematology, hormone assay and biochemistry
- Record the score of MENQOL and Greene Scale

Baseline / Visit 1

Procedure/assessments that must occur during the Baseline Visit include:

- Medical history
- Record the severity and frequency of vasomotor symptoms
- Randomize the patient (i.e. Baseline Visit only).
- Record body mass index and vital signs.
- Record the score of MENQOL and Greene Scale
- Record concomitant medications
- Dispense medication

Visit 2, Day 31

- Medical history
- Record the severity and frequency of vasomotor symptoms
- Record body mass index and vital signs.

- Obtain samples for hematology, hormone assay and biochemistry analysis
- Collect specimen for urinalysis.
- Record the score of MENQOL and Greene Scale
- Record adverse events and concomitant medications
- Dispense medication
- Medication accountability

Visit 3, Day 92

- Medical history
- Record the severity and frequency of vasomotor symptoms
- Record body mass index and vital signs.
- Obtain samples for hematology, hormone assay and biochemistry analysis
- Collect specimen for urinalysis.
- Record the score of MENQOL and Greene Scale
- Record adverse events and concomitant medications.
- Medication accountability

Follow-up period / Visit 4/ Day 120

- Medical history
- Record the severity and frequency of vasomotor symptoms
- Record body mass index and vital signs
- Obtain samples for hematology, hormone assay and biochemistry analysis
- Collect specimen for urinalysis
- Record the score of MENQOL and Greene Scale
- Record adverse events and concomitant medications
- Medication accountability

6.7 Clinical Laboratory Tests

Biochemistry, Hematology, Hormone Assay and Cardiovascular markers

The laboratory assessments will be performed in the Prince of Wales Hospital.

Appendix 2

The following will be performed:

Hematology:	Hemoglobin WBC count Platelets
Biochemistry:	Glucose Creatinine Albumin Plasma Urea ALP ALT/GPT ALT Total bilirubin Sodium Potassium
Urinalysis:	Glucose Protein acetone (ketone), pH
Hormone assay	FSH LH E2

6.8 Patient Medication Diary Card

The Patient Medication Diary Card will be issued to each patient at every visit starting from the Baseline Visit. Patients will be required to record the date of the use of the study medication during the study. The data collected from the patient's diaries will be

collected at each visit and the compliance will be checked by counting the remaining tablets/capsules.

6.9 Premature Discontinuation

6.9.1 Premature Withdrawal of Patients from the Study

Subjects must be withdrawn from the study if the subject withdraws consent. The withdrawal of consent may take place at any time; no justification for such a decision is required. Subjects will be encouraged to complete the study; however, they may voluntarily withdraw at any time. Nevertheless, if the subject withdraws from the study, a Premature Termination Visit should be made within 3 days after the last dose of the study medication.

A subject may discontinue taking the study medication for the following medical or administrative reasons, but will be followed per protocol until the end of the study:

- Adverse event(s) deemed sufficiently serious to require discontinuation of study medication
- At the discretion of the investigator
- Violation of eligibility criteria
- Deviation from the treatment plan specified in the protocol
- Progression of disease which, in the opinion of the principal investigator, should preclude further study
- Clinically significant intercurrent therapy which is thought to interfere with the outcome of this study
- Patient non-compliant with the study protocol.
- Patient lost to follow-up.

6.9.2 Premature Termination of Study/Closure of Study Site

Conditions that may warrant termination of the study include, but are not limited to:

- The discovery of an unexpected, serious, or unacceptable risk to the patients enrolled in the study.
- The decision to suspend or discontinue testing, evaluation, or development of the test agent.

7. STATISTICAL ANALYSIS

Sample size is selected based on feasibility. The primary endpoints of the study are vasomotor symptoms (hot flashes, night sweats and sweating), safety and tolerability.

Data will be processed to give group mean values and standard deviations where appropriate.

Thereafter each continuous variable will be tested for homogeneity of variance with Bartlett's test. If the variance is homogeneous, analysis of variance will be carried out for the variable. If any significant difference is detected, possible intergroup differences will be assessed with Dunnett's test. If the variance is heterogeneous, each variable will be tested for normality by the Shapiro-Wilk method. In case of normal distribution, t-test will be used to compare the difference between the two groups of treatment. Otherwise the non-parametric test of Mann-Whitney U-test will be used.

Group differences with an error probability of less than 5% ($p<0.05$) will be considered statistically significant.

The statistical analyses will be made with SPSS 11.3 for windows.

8. Safety Management

8.1 Definition of Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product. It does not necessarily have a causal relationship with this treatment.

An adverse event (AE) can therefore be an unfavorable and unintended sign (that

could include a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical product, whether or not considered related to the medical product.

An adverse event **does** include:

- An exacerbation of a pre-existing illness
- An increase in frequency or intensity of a pre-existing episodic event or condition
- A condition detected or diagnosed while on a study, which may have been present prior to the start of the study
- Continuous persistent disease/symptoms present at baseline that worsen following the start of the study

An adverse event **does not** include:

- Medical or surgical procedures (e.g. surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an AE
- Pre-existing disease or conditions present or detected at the start of the study that do not worsen
- Situations where an untoward medical occurrence has not occurred (e.g. hospitalizations for cosmetic elective surgery, social/convenience admissions)
- The disease being studied or signs/symptoms associated with the disease unless more severe than expected for the patient's condition
- Overdose of study medication or concurrent medication without any signs or symptoms

The intensity of each adverse event will be graded on a three point scale (mild, moderate and severe) and reported in detail as indicated on the Case Report Form.

Appendix 2

Mild: Adverse event usually transient in nature and generally not interfering with normal activities.

Moderate: Adverse event, which is sufficiently discomforting to interfere with normal activities.

Severe: Adverse event, which is incapacitating and prevents normal activities.

8.2 Definition of Adverse Reaction

All noxious and unintended responses to a medicinal product related to any dose will be considered adverse reactions.

8.3 Definition of Serious Adverse Event

In order to standardize investigator evaluation of safety, the use of the following standard assessment for the variable seriousness, labeling, intensity, outcome, and causality is imperative:

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Is fatal (results in death)
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Requires inpatient hospitalization
- Prolongs existing inpatient hospitalization

8.4 Adverse Event(s) Reporting

All adverse events must be fully recorded on the Adverse Event Page of the Case Record Form (CRF). Documentation must be supported by an entry in the patient's medical record.

Laboratory test abnormalities considered by the Investigator to be clinically relevant will be reported on the Adverse Event Page of the CRF.

Appendix 2

Signs and symptoms of each adverse event will be described in detail: date of onset, intensity, outcome, date of resolution, action taken with the study function, relationship to the study TCM and the seriousness of the adverse event.

8.5 Serious Adverse Event(s) Reporting

All serious adverse event whether or not believed to be drug-related must be reported to the Clinical Research Ethics Committee (CREC) and CCTCM, The Chinese University of Hong Kong by FAX (or telephone) within 24 hours upon the discovery of the event.

The Investigator is responsible to complete required SAE forms, report any SAE to the Clinical Research Ethics Committee (CREC) and respond promptly to any requests from the CREC for follow-up information.

A standard SAE from provided by the CREC and other SAE forms designed by the investigator will be forwarded to the CREC by fax. A Common Element Form (CEF) may be submitted to CREC by fax or e-mail.

CCTCM will review the information and contact the investigator for additional information or documentation, if necessary.

8.6 Clinically Significant Abnormalities

Laboratory analysis is frequently performed in the clinical trial. An abnormal laboratory finding is not by itself considered to be an AE or a serious AE (SAE) unless the investigator thinks the abnormal finding is of clinical significance and should be reported in such a manner. The abnormal laboratory finding does not have to be associated with the use of the test agent to be considered clinically significant. Those experiences that require special reporting are described above.

It is the responsibility of the Investigator to review and assess the clinical significance of all abnormal laboratory values as defined by the list of normal values. All

abnormal laboratory tests that are judged to be at least possibly treatment related or of uncertain causality must be repeated. For any significant changes noted by the investigator to be clinically significant or those that meet the criteria above, the clinical significance and relationship to the administration of the study treatment will be established. This assessment will be recorded on the CRF. If the changes are clinically significant, the patient will stop taking the study medication immediately. If the abnormal laboratory values do not return to normal within 14 days, the patient should be withdrawn from the study. In this case, the investigator will continue to monitor the patient until the parameter returns to normal or until the investigator determines that follow-up is no longer medically necessary.

8.7 Follow-up of Serious Adverse Event(s)

All SAEs must be followed until resolution or until the condition stabilizes or until the event is otherwise explained. A follow-up report which is to include a SAE summary and any new/updated information, will be faxed to the CREC within 15 working days after be alerted of the initial SAE.

The investigator will respond promptly to request for follow-up information from the CREC.

8.8 Ethics Committee Notification of Serious Adverse Event(s)

The Investigator is responsible for promptly notifying the local ethics committee of all serious adverse events, including follow-up information.

8.9 Notification of CCTCM of Post-Study Adverse Event(s)

The Investigator will notify the CCTCM of any death or serious adverse event

occurring at any time after a subject has signed out of a clinical trial, when such death or adverse event may reasonably be related to the treatment used in the study. Investigators are not obligated to actively seek adverse events in former study participants.

8.10 Breaking the Treatment Code due to AE(s) and SAE(s)

An emergency envelope containing the randomization code will be printed for each subject and must not be broken except for emergency situations. The investigator must inform the CCTCM immediately of any broken code. Any non-blinding of the study medication must be documented and explained by the investigator in the CRF.

9. INVESTIGATOR OBLIGATIONS

9.1 Data Collection

The investigator will maintain the individual patient files separate from the Case Record Form (CRFs). The individual patient files are considered source data and will include: visit dates of the patient, date of informed consent, date of randomization, medical history, physical examination, laboratory results, concomitant medications and treatments, any adverse events encountered and other notes as appropriate. All entries on the CRFs must be backed up by source data.

9.2 Study Records and Source Documents

Source documents may include subject's medical records, hospital charts, clinic charts, the Investigator's study files, as well as the results of diagnostic tests. The Investigator's copy of the CRF serves as the Investigator's record of a subject's study-related data.

All study-related correspondence, subject's records, consent forms, records of the distribution and use of all study medication, and copies of the CRFs will be maintained by the Investigator on file for at least two years after marketing approval

by the local regulatory authority. The direct access to these documents will be permitted in trial-related monitoring, audits, CREC review and regulatory inspection(s).

9.3 Disclosure

Individual subject medical information obtained as a result of this study is considered confidential, and disclosure to third parties other than those noted below is prohibited Such medical information may be given to the subject's personal physician or to other appropriate medical personnel responsible for the subject's welfare. Data generated as a result of this study will be made available for inspection upon request of the ethics committee or regulatory agency.

10. REFERENCES

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Appendix 3

PATIENT INFORMATION

Protocol Number: ICM/CTS/004

A Randomized, Double-Blind, Placebo-Controlled Study of the Effect of Danggui Preparation (當歸古方) on Menopausal Symptoms and Quality of life in Hong Kong Chinese Women

Sponsor: Institute of Chinese Medicine
The Chinese University of Hong Kong
2/F, Sciences Centre East Block
Shatin, N. T., Hong Kong

Patient Initial: | | | | (F M L)

Patient Number: | | | |

Research

You are invited to join a study of a Herbal Capsule for the treatment of menopausal symptoms. The name of the Herbal Capsule is Danggui Preparation (當歸古方). This patient information leaflet describes this clinical study in detail, which will help you to decide whether or not to join this study. Please read through it carefully and do not hesitate to ask if you have any doubts or queries. Joining this study is voluntary. If you decide to join this study, you will be asked to sign a consent form.

Why conduct this study?

The purpose of this study is to examine the effectiveness and safety of this Herbal Capsule in treating menopausal symptoms and to examine its effect on quality of life. All participants will be randomized to receive study medication or placebo (inactive treatment) for a period of 6 months.

What is included in this study?

This study will only be conducted in Hong Kong. About 100 patients will be recruited into this study. Each participant will join this study for a period of 6 months.
This study has been approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong.
If you agree to participate in the study, you will need to visit this clinic 4 times during the study period for medical assessments. Blood samples will be obtained at each visit.
If you decide to join this study, you will be randomly assigned to one of the two groups:

Group 1: Will take the Herbal Capsules daily for 6 months.

Group 2: Will take the placebo (non-active) capsules daily for 6 months.

During the treatment period, neither you nor your doctor will know which treatment/group you are assigned to. This is standard procedure in performing a clinical study and will ensure that the investigating doctor makes a fair and objective observation/assessment. However, in an event of emergency, the doctor is able to find out immediately which treatment/group you are assigned to.

Study Procedure

This study is basically divided into 2 different periods: screening period and treatment period.

After your informed consent has been obtained, you will be screened by the doctor to determine if you are eligible to participate in the study. The screening will include asking about your previous medical history, carrying out a physical examination, measuring your heart rate, blood pressure and vital signs. Your height, weight and any concomitant medications will be recorded. Blood samples will be collected for analysis including hematology, biochemistry, hormone assay and cholesterol profile. A sample of urine will be collected for standard laboratory tests. The screening phase will last up to 30 days before the start of treatment. If you are eligible for this study, you will enter into the treatment phase and be scheduled for visits at day 0 (Baseline), day 91 and day 182. The study medication will also be delivered to you after the baseline visit and thereafter at each visit.

Any unused medication will be collected at each appointment.

How should I take the study medication?

The treatment will start at in the week of the Baseline visit. You will need to take the study medication orally 3 times a day. You must take the capsules as scheduled and record the time and date in the patient medication diary card. At each visit, your diary card will be collected and a new diary card will be dispensed.

If you experience any serious adverse reaction, the investigating doctor may temporally or permanently stop your treatment.

The study medication provided to you in this clinical study is only used for studying its effects on menopausal symptoms and should not be used for other purposes. You should not give these study medications to others. The study medication should not be accessible to children.

You should consult your investigating doctor before taking any other medication.

What are the risks?

All drugs have the potential to cause side effects in some patients. Although the toxicity tests to date with Danggui Preparation (當歸古方)do not show any unfavorable safety results, there is a possibility of side effects.

During every visit, the investigator will assess you according to the parameters set forth for close monitoring of side effects.

The risks of blood drawing are minimal but do include the temporary pain of the needle stick, occasional bruising and rarely inflammation of the vein. A small amount of the blood will be drawn at Screening, Visit 2 and Visit 3.
Your doctor may terminate your participation without your consent if you have unacceptable side effects or adverse reactions. Any significant findings discovered during this study which may relate to your condition will be provided to you.

What are the benefits?

If Danggui Preparation (當歸古方) is proved to be effective, your menopausal symptoms will be treated/controlled. In addition, knowledge on treating menopausal symptoms with Danggui Preparation (當歸古方) will be increased because of the data obtained by your involvement in the study. It is hoped that this treatment will also improve quality of life. This information may help other patients with menopausal symptoms. You will not be provided with any of the study medication after the termination of this study and therefore you may need to discuss with your doctor alternate treatment regimens.

Any other alternative treatment regimens?

Currently, there are a number of treatment regimens available for treating menopausal symptoms. These include Estrogen Replacement Therapy. These are available in Hong Kong and many other countries.

Will my results be kept confidential?

Your investigating doctor will record the results of all the tests on a Case Record Form (CRF) during each of your visits. Your participation in the study will be treated as confidential and any records or results relating to the trial shall not be disclosed to any third party other than a representative of the sponsor or governmental medical regulatory bodies. By signing the consent form, you authorize direct access to your medical records for the purpose of monitoring, auditing and inspection or as may otherwise be required by law. Only your initials and your patient number will appear in the study document. Information obtained in the study may be used in publications or reports but you will not be referred to by name.

If there are problems...

You will not need to pay for taking part in this study. The sponsor will pay all the study costs. However, costs for regular medical care, which are not related to this study, will be your own responsibility. No compensation is available for lost wages and/or pain and suffering. By signing this consent form, you have not waived any of the legal rights which you otherwise would have as participant in a research study.

If I change my mind...

The decision to join this study is entirely voluntary. You can refuse to participate or withdraw from the study at any time without penalty or loss of any benefit to which you are otherwise entitled. Refusal or withdrawal will not influence your routine medical care.

However, if you decide not to participate in this study after you have taken the study medication, you should go through a termination study procedure as specified by your study doctor within 7 working days after your last dose of study medication. You do not need to give any reasons for withdrawing from this study, but you should inform your doctor, especially if your reason for withdrawal is related to side effects.

If you cannot follow the procedures and requirements of this study or your investigating doctor thinks that withdrawal from this study is in your best interests, he has the authority to withdraw you from this study.

If I have other questions...

If you have any questions related to this study or believe that due to study participation you have sustained an injury or had adverse reaction to this study medication, at any time please contact Dr. Leung Pui Ling at Tel No.: 26322810.

Appendix 3

病人須知

方案編號: ICM/CTS/004

研究題目：當歸古方對治療香港華籍更年期婦女症狀的療效及其對生活質素之影響的隨機雙盲對照研究

研究者　　威爾斯親王醫院
香港中文大學婦產科學系
新界香港中文大學

病人英文姓名字首

F	M	L

病人編號

我們現誠邀閣下參與一項使用中藥膠囊以治療更年期症狀的臨床研究，該中藥的名稱爲當歸古方。這份病人須知將爲你詳細描述這項研究的有關資料，以幫助你決定是否參與。請仔細閱讀整份病人須知，如有任何疑問，請向研究助理提出，而參與這項研究與否純屬自願性質，如果你決定參與，你需要簽署一份同意書。

爲什麼要進行這項研究？

這項研究的目的是觀察當歸古方對治療更年期症狀的療效及其安全性，以及探討這中藥對病人生活質素的影響。所有參與這項研究的病人，將被隨機安排接受爲期六個月的治療。

這項研究包括什麼？

這研究只會在香港進行，我們計劃招募大約 100 位病人，每位病人將會參與這項研究六個月。

這項研究已獲得香港中文大學的臨床研究道德倫理委員會的批准。

如果閣下同意參與這項研究，你便需要在研究治療期間，按時回來覆診四次，及作身體檢查。在四次覆診中你亦會接受抽取血液樣本作相關測試，用以評價治療效果。

你在加入這項研究後，將會被隨機分配到以下兩個治療組別的其中一個。

第一組　每天服用當歸古方膠囊，爲期六個月。

第二組　每天服用安慰劑膠囊(無藥性成分)，爲期六個月。

在研究期間，你及你的醫生均不會知道你接受的是哪一組別的研究藥品。這是臨床試驗的慣常程序，這項程序可以確保研究醫生所作出的觀察及評估是公平及客觀的。不過當發生緊急情況時，你的研究醫生可因應情況需要找出你正在接受的是哪一組別的研究藥品。

Appendix 3

研究程序

這項研究基本上可分爲二階段 甄選期和治療期。

當你在這份同意書上簽署後，醫生會對你的身體狀況作出評估，以決定你是否適合參與這項研究。甄選的程序包括詢問你的病歷，替你進行一次全面的身體檢查，測量你的心跳、血壓、身體表徵、身高、體重，及記錄你服用藥物的情況。此外，我們將會抽取你的血液作血液學、血液生化、荷爾蒙水平和血脂的測試。你的尿液樣本亦會被收集作標準的測試。這段甄選期爲期三十日，直到治療開始爲止。如你適合參與這項研究，你將會進入治療的階段，並需在第 0 日(基礎檢驗)，第 91 日，及第 182 日回診所覆診。

任何剩下或沒有服用的研究藥品均需要在每次覆診時交回。

我應怎樣服用研究藥品 ?

研究藥品 (當歸古方或安慰劑) 的治療會在基礎檢驗後 (即第 1 天) 開始。 每天你需要口服研究藥品二次。你需要根據時間表服用並需要在病人服藥日誌上記錄服用的時間及日期。當你每次覆診時，你的病人服藥日誌將會被收回，而一張新的病人服藥日誌亦會被分發給你。

如果在治療期間發生嚴重的不良反應，醫生會暫時或永久性地停止你的療程。

這項研究提供的研究藥品只作研究更年期症狀使用，而不可作其他用途。你亦不應將研究藥品給予他人使用。研究藥品應儲存於室溫及兒童不能觸及之處。

在開始服用任何新藥之前，你應諮詢你的研究醫生。在研究期間，你應避免濫用酒精或其他藥物，因爲這些都會影響研究藥品的效果。

有什麼風險 ?

任何藥物均有可能在某一些病人身上引起副作用。雖然當歸古方是一純中藥配方，其安全性已得到証實，在醫生的指導下使用是安全的，但我們仍不能排除它可能引起一些目前尚未瞭解的不良影響。

在每次覆診時，研究人員會爲你進行評估，而這些評估是爲了密切監察所有副作用而設的。

抽血的風險是極微的，但仍包括抽血部位疼痛，瘀傷及不常發生的靜脈發炎。在基礎檢驗，第二及第三次覆診時，你需要接受抽取少量血液，以作相關測試。

如果你出現一些無法接受的副作用或不良反應，你的研究醫生可能會在未得到你的同意下，終止你的參與。在研究期間，若發現任何影響你的情況，研究醫生會立即通知你。

有何利益 ?

我們不能保證你參與這項研究能得到任何益處。但是如果當歸古方被證實有好的療效，你的更年期症狀會被治愈或控制。此外，因爲你的參與而獲得的資料將會增加我們對當歸古方被用作治療更年期症狀的認識。這些資料均能幫助其他有更年期症狀的病人。研究結束後我們不會再提供研究藥品，所以在研究結束時，如有需要，你應與你的醫生討論其他治療方案。

有哪些其他治療方案 ?

現時治療更年期症狀的方案有很多，如雌激素替代療法。採用雌二醇也是治療方法之一。它們均在香港及其他國家有供應的。

Appendix 3

我的結果會被保密嗎？

你的研究醫生會把你每次覆診所接受的測試結果記錄下來。這些結果會被記錄在病例記錄表(CRF) 上。你參與這項研究的資料將會被保密，任何與這項研究有關的紀錄或結果亦不會泄露給除研究者代表或政府醫學管理的團體之外的第三者。簽署這份同意書後，表示你授權直接使用你的醫療記錄作法律許可的監察，審核及檢查的用途。只有你的姓名字首及研究開始時被編排的病人編號會在研究的文件上出現。這項研究中獲得的資料可能會被公佈或報告，但你的名字並不會被提及。

如果有問題出現時...

你不需要因參與這項研究而支付任何費用。研究者將會支付這項研究的一切費用。但任何與這項研究無關的日常醫療費用，你則需要負責。此外，任何因這項研究過程引致的工資損失、疼痛或受到的痛苦均不會獲得賠償。簽署這份同意書後，你並沒有放棄任何作爲研究參與者應有的法律權利。

如果我改變主意...

參與這項研究完全是自願性質。你可拒絕參與或退出這項研究，而你亦不會因此遭受任何處罰或失去你應有的權益。此外,亦不會影響你常規的醫療護理。

但是，如果你在服用研究藥品後退出，你應在服用最後一劑研究藥品後七個工作天內進行一項終止研究的程序。你無須說明退出理由，但你必需通知你的醫生，尤其如果你是因爲副作用而決定退出的。

如果你不能依照研究程序的要求或你的研究醫生因任何原因認爲你退出研究比較恰當，他亦有權讓你退出這項研究。

如果我有其他問題...

如你有任何關於這項研究的問題或你認爲你已因爲參與這項研究而受傷或對研究藥品有不良反應，你可聯絡梁佩玲醫生，電話 2632 2810。

Appendix 3

方案編號: ICM/CTS/004

當歸古方對治療香港華籍更年期婦女症狀的療效及其對生活質素之影響的隨機雙盲對照研究

我_____(病人姓名)已明瞭上述研究的資料及該研究的性質,目的及其可能帶來的益處和風險。我明白我將會收到一份我簽署的同意書副本。我亦明白我的醫生或研究者可能在未得到我的同意下終止我參與這項研究。我瞭解我並沒有義務必須參與這項研究, 以及我可在任何時間退出而不需要說明任何理由, 而我將來接受的醫療護理亦不會被影響。我已有充足的時間決定是否參與。

作爲研究的參加者, 我明白我的醫療記錄將會被研究人員或其他授權人員在保密的情況下使用。

我特此確認並同意上述條款，自願參與這項研究。

_____ _____

病人簽署 簽署日期

_____ _____

見證人簽署 簽署日期

醫生/ 研究醫生聲明 我已向參與者說明這項研究的性質。我特此證明參與者在簽署這份同意書時已清楚瞭解這項研究的性質, 要求, 風險及益處, 而身體, 語言或教育的障礙並沒有妨礙參與者瞭解這項研究。

_____ _____

醫生/ 研究醫生簽署 簽署日期

Appendix 3

PATIENT INFORMED CONSENT

Protocol Number: ICM/CTS/004

A Randomized, Double-Blind, Placebo-Controlled Study of the Effect of Danggui Preparation (當歸古方) on Menopausal Symptoms and Quality of Life in Hong Kong Chinese Women

I_____(Patient Name) have obtained adequate information about the above study and understand its nature, purpose, possible benefits and potential risks. I understand I will receive a copy of my signed consent form. I also understand that my doctor and the sponsor organization can stop my participation in this study without my consent. I have no obligation to participate and can withdraw at any time without any reason and my medical care will not be affected in future. I have also been given enough time to decide whether to participate or not.

As a participant in the study, I understand that my medical records will be accessible to a research monitor or other authorized persons but the records will be kept confidential.

I hereby confirm my agreement to the above and to participate voluntarily in this study.

_____ _____
Signature of Participant Date of Signature

_____ _____
Signature of Witness Date of Signature

Physician/Investigator Statement: The nature of the study has been carefully explained to the participant. I hereby certify that to the best of my knowledge the participant signing this consent form understands clearly the nature, demands, risks and benefits involved in participating in this study. A medical problem or language

or educational barrier has not prevented a clear understanding of the participant's involvement in this study.

Signature of Physician/Investigator	Date of Signature

Date of Signature

Appendix 4

Protocol Code	Screening Number	Subject Initials	Subject Number
ICM/CTS/05/336		F M L	

CASE REPORT FORM

A Randomized, Double-Blind, Multiple-Dose Escalation Study of the Effect of Danggui Buxue Tang (DBT 當歸補血湯) on symptomatic postmenopausal Hong Kong Chinese Women
(Continuation of a completed study using the same formula)

Version: 4

Date: September 2005

Department of Obstetrics & Gynaecology
Centre for Clinical Trials on Chinese Medicine
The Chinese University of Hong Kong
Prince of Wales Hospital
Shatin, N T , Hong Kong

I confirm that the information given in this case report form is accurate and complete

Signed _____ Date D D M M Y Y

Printed Name _____

Protocol Code	Subject Initials	Screening Number	Visit Date
ICM/CTS/05/336	F M L		D D M M Y Y

SCREENING

Inclusion Criteria *MUST BE CHECKED **YES** TO PERMIT STUDY PARTICIPATION*

Does the patient meet all the following inclusion criteria? (Check YES or NO for all below)

		YES 1	NO 2
1	Is the Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) Oestradiol of the patient in the menopausal range (FSH>18IU/L, LH>12 6IU/L and E2< 361pmol/l)?	☐	☐
2	Is the patient with menopausal amenorrhoea more than 12 months?	☐	☐
3	Does the patient never receive treatment for menopausal symptoms?	☐	☐
4	Does the patient never receive menopausal hormone therapy?	☐	☐
5	Is the patient with at least 14 hot flushes per week at the time of entry into the study?	☐	☐

Date Consent Form Signed ☐☐☐☐☐☐
D D M M Y Y

Protocol Code	Subject Initials	Screening Number	Visit Date
ICM/CTS/05/336	F M L		D D M M Y Y

SCREENING

Exclusion Criteria *MUST BE CHECKED **NO** TO PERMIT STUDY PARTICIPATION*

Does the patient demonstrate any of the following exclusion criteria? (Check YES or NO for all below)

		YES 1	NO 2	NA 3
1	Does the patient use Chinese Medicine or other therapies which may affect the outcomes within 8 weeks?	☐	☐	
2	Is the patient, in the judgement of the Investigator, unable to comply with the protocol requirements?	☐	☐	
3	Is the patient with significant** gastrointestinal, renal, hepatic, bronchopulmonary, neurological, cardiovascular, breast or endometrial carcinoma, or allergy diseases?	☐	☐	
4	Is the patient with uncontrolled hypertension?	☐	☐	
5	Is the patient with undiagnosed vaginal bleeding?	☐	☐	

** Significant is defined as a disease condition that requires hospitalization or prolongs the hospitalization within the past 2 years

Protocol Code	Subject Initials	Screening Number	Visit Date
ICM/CTS/05/336	F M L		D D M M Y Y

SCREENING

Demographics

Date of Birth ☐☐☐☐☐☐ (D D M M Y Y)

Weight ☐☐☐ . ☐ kg

Height ☐☐☐ . ☐ cm

Medical History

Indication Findings	Yes 1	No 2	UNK 3	Specify any Abnormalities, and comment.
1 Cardiovascular Disease	☐	☐	☐	
2 Bronchpulmonary Disease	☐	☐	☐	
3 Gastrointestinal Disease	☐	☐	☐	
4 Genitourinary Disease	☐	☐	☐	
5 Metabolic / Endocrine Disease	☐	☐	☐	
6 Hematopoietic Disease	☐	☐	☐	
7 Musculoskeletal Disease	☐	☐	☐	
8 Neurological Disease	☐	☐	☐	
9 Psychiatric Disease	☐	☐	☐	
10 ENT Disease	☐	☐	☐	
11 Malignancy, Cancer	☐	☐	☐	
12 Other	☐	☐	☐	
13 Other	☐	☐	☐	

Protocol Code	Subject Initials	Screening Number	Visit Date
ICM/CTS/05/336	F M L		D D M M Y Y

SCREENING

Vital Signs

Temperature ☐☐☐.☐ ☐1 C ☐2 F

Physical Examination

Indication Findings	Normal 1	Abnormal 2	Not Examined 3	Specify any Abnormalities, and comment.
1 General Appearance	☐	☐	☐	
2 ENT	☐	☐	☐	
3 Chest / Lungs	☐	☐	☐	
4 Liver / Spleen	☐	☐	☐	
5 Lymph Nodes	☐	☐	☐	
6 Abdomen	☐	☐	☐	
7 Skin	☐	☐	☐	
8 Musculoskeletal	☐	☐	☐	
9 Extremities	☐	☐	☐	
11 Other		☐		

Protocol Code	Subject Initials	Screening Number	Visit Date
ICM/CTS/05/336	F M L		D D M M Y Y

SCREENING

Blood Pressure and Heart Rate

Time of measurement ☐☐ : ☐☐ (24 hour clock)

Measurement	Systolic (mm Hg)	Diastolic (mm Hg)
Blood Pressure	☐☐☐	☐☐☐

Heart Rate (beats / min)	☐☐☐
Body Mass Index (kg/m^2)	☐☐ ☐

Laboratory Tests

Date of Blood Drawing ☐☐☐☐☐☐ (D D M M Y Y)

Protocol Code	Subject Initials	Subject Number	Visit Date
ICM/CTS/05/336	F M L		D D M M Y Y

SCREENING

Laboratory Tests

List below all laboratory findings

Attached the laboratory report to this page

Time Specimens Obtained ☐☐ : ☐☐ (24-hour clock)

		Result
Hematology	Hemoglobin	
	Erythrocytes	
	White Blood Count	
Biochemistry	Albumin	
	Calcium	
	Alkaline Phosphates	
	Phosphatase Inorganic	
	ALT/GPT	
	Bilirubin	
	Total Protein	
	Creatinine	
	Potassium	
	Sodium	
	Glucose	
	Urea	

		Result		
		neg	trace	pos
Urinalysis	Protein	☐	☐	☐
	Glucose	☐	☐	☐
	Protein Acetone (Ketone)	☐	☐	☐
Hormone	FSH			
	LH			
	E2			
Lipids	CHOL			
	LDL-C			
	HDL-C			
	TG			

Specific Examination

Test	Normal	Abnormal	Result
Pelvic Ultrasound	☐	☐	

Comments on results _____

Protocol Code: **ICM/CTS/05/336**

Subject Initials: F M L

Screening Number

CONCOMITANT MEDICATION-----Screening

Please record all current and previous (within past 60 days prior) medications

Concomitant Medication 1 Yes 2 No

Drug name (Enter Generic name)	Indication (Use standard medical terminology)	**Start Date (if known) and Stopped Date** (DD-MM-YY)	Check if Continuing	Unit Dose (e g , 10mg, 10 units)	Frequency*	Route** (enter code)	Was drug administered for prophylaxis
1		Start Date Stop Date	1C				1 Yes 2 No
2		Start Date Stop Date	1C				1 Yes 2 No
3		Start Date Stop Date	1C				1 Yes 2 No
4		Start Date Stop Date	1C				1 Yes 2 No
5		Start Date Stop Date	1C				1 Yes 2 No
6		Start Date Stop Date	1C				1 Yes 2 No

*Frequency 0=PRN 1=QD 2=BID 3=TID 4=QID 5=Other (specify)

**Route 1=IM 2=IV 3=PO 4=SC 5=Rectal 6=Topical 7=Nasal 8=Inhaled 9=Other (Specify)

停經後生活質素調查問卷(For Screening Use)

Page 1 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
☐☐☐	ICM/CTS/05/336	☐☐☐	☐☐ / ☐☐ / ☐☐ (DAY / MONTH / YEAR)

在下列各題中，請指出在上個月有否經歷有關情況。如有，請按程度評分。

				從未因此煩惱							非常煩惱
						0	1	2	3	4	5	6	
1	潮熱	不【 】	【 】是	→	0	1	2	3	4	5	6	
2	夜汗	不【 】	【 】是	→	0	1	2	3	4	5	6	
3	易汗	不【 】	【 】是	→	0	1	2	3	4	5	6	
4	對個人生活不滿	不【 】	【 】是	→	0	1	2	3	4	5	6	
5	煩躁易怒	不【 】	【 】是	→	0	1	2	3	4	5	6	
6	記憶力差	不【 】	【 】是	→	0	1	2	3	4	5	6	
7	工作能力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
8	抑鬱感	不【 】	【 】是	→	0	1	2	3	4	5	6	
9	不耐煩與人相處	不【 】	【 】是	→	0	1	2	3	4	5	6	
10	希望或需要獨處	不【 】	【 】是	→	0	1	2	3	4	5	6	
11	胃腸脹氣或脹氣疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
12	肌肉或關節疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
13	疲勞感	不【 】	【 】是	→	0	1	2	3	4	5	6	
14	難以入眠	不【 】	【 】是	→	0	1	2	3	4	5	6	
15	頸痛或頭痛	不【 】	【 】是	→	0	1	2	3	4	5	6	

MENQOL-1

更年期生活質素調查問卷(For Screening Use)

Page 2 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
	ICM/CTS/05/336		☐☐ / ☐☐ / ☐☐ (DAY / MONTH / YEAR)

在下列各題中，請指出在上個月有否經歷有關情況。如有，請按程度評分。

					從未因此煩惱							非常煩惱
					0	1	2	3	4	5	6	
16	體力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
17	耐力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
18	精力匱乏	不【 】	【 】是	→	0	1	2	3	4	5	6	
19	皮膚乾燥	不【 】	【 】是	→	0	1	2	3	4	5	6	
20	體重增加	不【 】	【 】是	→	0	1	2	3	4	5	6	
21	面毛增加	不【 】	【 】是	→	0	1	2	3	4	5	6	
22	皮膚外觀、質感和彈性改變	不【 】	【 】是	→	0	1	2	3	4	5	6	
23	感覺腸胃氣脹	不【 】	【 】是	→	0	1	2	3	4	5	6	
24	腰背疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
25	尿頻	不【 】	【 】是	→	0	1	2	3	4	5	6	
26	大笑或咳嗽時容易尿失禁	不【 】	【 】是	→	0	1	2	3	4	5	6	
27	性慾改變	不【 】	【 】是	→	0	1	2	3	4	5	6	
28	性交時陰道乾燥	不【 】	【 】是	→	0	1	2	3	4	5	6	
29	逃避親密行爲	不【 】	【 】是	→	0	1	2	3	4	5	6	

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Protocol Code	Subject Initials	Subject Number	Visit Date
ICM/CTS/05/336	F M L		D D M M Y Y

Baseline: Visit 1 / Day 0

Vital Signs

Temperature (C^0) ☐☐.☐ Weight (kg) ☐☐☐.☐

Blood Pressure, Heart Rate and Body Mass Index

Time of measurement ☐☐ : ☐☐ (24 hour clock)

Measurement	Systolic (mm Hg)	Diastolic (mm Hg)
Blood Pressure	☐☐☐	☐☐☐

Heart Rate (beats / min)	☐☐☐
Body Mass Index (kg/m^2)	☐☐.☐

Dispense Study Medication

Date of Issue ☐☐☐☐☐☐ (D D M M Y Y) Dosage Issued ☐☐ Sachets

停經後生活質素調查問卷(第 1次見面)

Page 1 of 2

SUBJECT NO	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
	ICM/CTS/05/336		☐☐ / ☐☐ / ☐☐ DAY / MONTH / YEAR

在下列各題中，請指出在上個月有否經歷有關情況。如有，請按程度評分。

					從未因此煩惱							非常煩惱
							0	1	2	3	4	5	6	
1	潮熱	不【 】	【 】是	→	0	1	2	3	4	5	6	
2	夜汗	不【 】	【 】是	→	0	1	2	3	4	5	6	
3	易汗	不【 】	【 】是	→	0	1	2	3	4	5	6	
4	對個人生活不滿	不【 】	【 】是	→	0	1	2	3	4	5	6	
5	煩躁易怒	不【 】	【 】是	→	0	1	2	3	4	5	6	
6	記憶力差	不【 】	【 】是	→	0	1	2	3	4	5	6	
7	工作能力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
8	抑鬱感	不【 】	【 】是	→	0	1	2	3	4	5	6	
9	不耐煩與人相處	不【 】	【 】是	→	0	1	2	3	4	5	6	
10	希望或需要獨處	不【 】	【 】是	→	0	1	2	3	4	5	6	
11	胃腸脹氣或腹氣疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
12	肌肉或關節疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
13	疲勞感	不【 】	【 】是	→	0	1	2	3	4	5	6	
14	難以入眠	不【 】	【 】是	→	0	1	2	3	4	5	6	
15	頸痛或頭痛	不【 】	【 】是	→	0	1	2	3	4	5	6	

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更年期生活質素調查問卷(第 1次見面)

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SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
	ICM/CTS/05/336		☐☐ / ☐☐ / ☐☐ DAY / MONTH / YEAR

在下列各題中，請指出在上個月有否經歷有關情況。如有，請按程度評分。

					從未因此煩惱							非常煩惱
					0	1	2	3	4	5	6	
16	體力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
17	耐力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
18	精力匱乏	不【 】	【 】是	→	0	1	2	3	4	5	6	
19	皮膚乾燥	不【 】	【 】是	→	0	1	2	3	4	5	6	
20	體重增加	不【 】	【 】是	→	0	1	2	3	4	5	6	
21	面 毛增加	不【 】	【 】是	→	0	1	2	3	4	5	6	
22	皮膚外觀、質感和彈性改變	不【 】	【 】是	→	0	1	2	3	4	5	6	
23	感覺腸胃氣脹	不【 】	【 】是	→	0	1	2	3	4	5	6	
24	腰背疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
25	尿頻	不【 】	【 】是	→	0	1	2	3	4	5	6	
26	大笑或咳嗽時容易尿失禁	不【 】	【 】是	→	0	1	2	3	4	5	6	
27	性慾改變	不【 】	【 】是	→	0	1	2	3	4	5	6	
28	性交時陰道乾燥	不【 】	【 】是	→	0	1	2	3	4	5	6	
29	迴避親密行爲	不【 】	【 】是	→	0	1	2	3	4	5	6	

MENQOL-1

MENOPAUSE QUALITY OF LIFE RECORD(Visit 1)

Page 1 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
	ICM/CTS/05/336		☐☐ / ☐☐ / ☐☐ (DAY / MONTH / YEAR)

FOR EACH OF THE FOLLOWING ITEMS, INDICATE WHETHER YOU HAVE EXPERIENCED THE PROBLEM IN THE **PAST MONTH.**
IF YOU HAVE, RATE HOW MUCH YOU HAVE BEEN **BOTHERED** BY THE PROBLEM.

					NOT AT ALL BOTHERED							EXTREMELY BOTHERED
					0	1	2	3	4	5	6	
1	HOT FLUSHES OR FLASHES	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
2	NIGHT SWEATS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
3	SWEATING	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
4	BEING DISSATISFIED WITH MY PERSONAL LIFE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
5	FEELING ANXIOUS OR NERVOUS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
6	EXPERIENCING POOR MEMORY	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
7	ACCOMPLISHED LESS THAN I USED TO	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
8	FEELING DEPRESSED, DOWN, OR BLUE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
9	BEING IMPATIENT WITH OTHER PEOPLE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
10	FEELING OR WANTING TO BE ALONE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
11	FLATULENCE (WIND) OR GAS PAINS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
12	ACHING IN MUSCLES AND JOINTS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
13	FEELING TIRED OR WORN OUT	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
14	DIFFICULTY SLEEPING	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
15	ACHES IN BACK OF NECK OR HEAD	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	

MENQOL-1

MENOPAUSE QUALITY OF LIFE RECORD(Visit 1)

Page 2 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
	ICM/CTS/05/336		☐☐ / ☐☐ / ☐☐ (DAY / MONTH / YEAR)

FOR EACH OF THE FOLLOWING ITEMS, INDICATE WHETHER YOU HAVE EXPERIENCED THE PROBLEM IN THE **PAST MONTH.**
IF YOU HAVE, RATE HOW MUCH YOU HAVE BEEN **BOTHERED** BY THE PROBLEM.

					NOT AT ALL BOTHERED						EXTREMELY BOTHERED
16	DECREASE IN PHYSICAL STRENGTH	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
17	DECREASE IN STAMINA	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
18	FEELING A LACK OF ENERGY	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
19	DRYING SKIN	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
20	WEIGHT GAIN	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
21	INCREASE IN FACIAL HAIR	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
22	CHANGES IN APPEARANCE, TEXTURE, OR TONE OF YOUR SKIN	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
23	FEELING BLOATED	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
24	LOW BACKACHE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
25	FREQUENT URINATION	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
26	INVOLUNTARY URINATION WHEN LAUGHING OR COUGHING	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
27	CHANGE IN YOUR SEXUAL DESIRE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
28	VAGINAL DRYNESS DURING INTERCOURSE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
29	AVOIDING INTIMACY	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6

MENQOL-1

DAILY RECORD - TO BE COMPLETED AT VISIT 1, VISIT 2 AND VISIT 3

Page 1 of 2

TABLETS STARTED ON: ___ ___ - ___ ___ - ___ ___ (DAY - MONTH - YEAR)

DAILY RECORD STARTED ON: ___ ___ - ___ ___ - ___ ___ (DAY - MONTH - YEAR)

SUBJECT NUMBER: _____

SUBJECT INITIALS: _____

TABLET TAKEN: ↓

Day of Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Tablets Taken: Enter 0 or 1																												

Hot Flush Definiations:

Mild = *Fleeting warm sensation, no sweating, does not disrupt activity*

Moderate = *Warm sensation, with sweating, does not disrupt activity*

Severe = *Hot sensation with sweating, disrupts activity*

Enter "0" = none or actual number of hot flushes by severity below.

Day of Cycle		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Hot Flushes	Mild																												
	Moderate																												
	Severe																												

Enter "0" = no or "1" = yes for each occurrence of the postmenopausal symptoms listed below.

Day of Cycle	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Night Sweats																												
Insomnia																												
Feeling Bloated																												
Vaginal Dryness or Irritation																												
Vaginal Itching																												
Painful Intercourse																												
Less Interest in Intimacy																												
Painful urination or urgency																												
Palpitations																												
Malaise																												
Body or Joint Aches/Pains																												
Mood Swings																												
Decline in Memory																												
Loss of Concentration																												
Nervousness/Irritability																												
Skin Texture Changes																												
Hair Texture Changes																												

DAILY RECORD - TO BE COMPLETED

TABLETS STARTED ON:

DAY ____ MONTH ____ YEAR ____

Protocol Code
ICM/CTS/05/336

SUBJECT NUMBER: _____

SUBJECT INITIALS: _____ (V.)

DAILY RECORD STARTED ON:

DAY ____ MONTH ____ YEAR ____

DA

Day of Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Tablets Taken: Enter 0 or 1																															

HF

Hot Flush Definiations:

Mild = *Fleeting warm sensation, no sweating, does not disrupt activity*

Moderate = *Warm sensation, with sweating, does not disrupt activity*

Severe = *Hot sensation with sweating, disrupts activity*

Enter "0" = no or "1" = yes for each occurrence of the postmenopausal symptoms listed below. *

Day of Cycle		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	Mild																															
Hot Flushes	Moderate																															
	Severe																															

NS

Enter "0" = none or "1" for Night Sweats.

Day of Cycle	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Night Sweats																															

DAILY RECORD STARTED ON:

DAY ____ MONTH ____ YEAR ____

DA

Day of Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Tablets Taken: Enter 0 or 1																															

HF

Hot Flush Definiations:

Mild = *Fleeting warm sensation, no sweating, does not disrupt activity*

Moderate = *Warm sensation, with sweating, does not disrupt activity*

Severe = *Hot sensation with sweating, disrupts activity*

Enter "0" = no or "1" = yes for each occurrence of the postmenopausal symptoms listed below.

日期		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	Mild																															
Hot Flushes	Moderate																															
	Severe																															

NS

Enter "0" = none or "1" for Night Sweats.

Day of Cycle	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Night Sweats																															

Please bring the Daily record with you when you visit the clinic

Appendix 4

每日記錄卡

服用沖劑的首日日期:

□□ - □□ - □□
日 月 年

Protocol Code
ICM/CTS/05/336

病人號碼: _____

病人姓名簡寫: _____ (V.)

_____月_____年

服藥	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
	已服用沖劑請填上「1」，如無則填上「0」																																

潮熱

潮熱定義：
輕微 = 感覺溫熱但不會出汗，並未影響起居生活
中度 = 感覺溫熱會出汗，並未影響起居生活
嚴重 = 感覺溫熱且出汗並影響起居生活

請依照上列嚴重程度，填上當天潮熱次數，如無則填上「0」。

日期		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
	輕微																																
潮熱：	中度																																
	嚴重																																

夜汗

如出現夜汗情況請填上「1」，如無則填上「0」。

日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
夜汗																																

_____月_____年

服藥	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
	已服用沖劑請填上「1」，如無則填上「0」																																

潮熱

潮熱定義：
輕微 = 感覺溫熱但不會出汗，並未影響起居生活
中度 = 感覺溫熱會出汗，並未影響起居生活
嚴重 = 感覺溫熱且出汗並影響起居生活

請依照上列嚴重程度，填上當天潮熱次數，如無則填上「0」。

日期		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
	輕微																																
潮熱：	中度																																
	嚴重																																

夜汗

如出現夜汗情況請填上「1」，如無則填上「0」。

日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
夜汗																																

每次覆診，請帶同此日記紙

Protocol Code	Subject Initials	Subject Number	Visit Date
ICM/CTS/05/336	F M L		D D M M Y Y

Visit 2 / Day 31

Vital Signs

Temperature (C^0) ☐☐.☐ Weight (kg) ☐☐☐.☐

Blood Pressure, Heart Rate and Body Mass Index

Time of measurement ☐☐ : ☐☐ (24 hour clock)

Measurement	Systolic (mm Hg)	Diastolic (mm Hg)
Blood Pressure	☐☐☐	☐☐☐

Heart Rate (beats / min)	☐☐☐
Body Mass Index (kg/m^2)	☐☐.☐

Dispense Study Medication

Date of Issue ☐☐☐☐☐☐ (D D M M Y Y) Dosage Issued ☐☐ Sachets

Medication Accountability

No of sachets returned ☐☐ Missing dose ☐☐

Reason _____

停經後生活質素調查問卷(第 2次見面)

Page 1 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
	ICM/CTS/05/336		□□ / □□ / □□ (DAY / MONTH / YEAR)

在下列各題中，請指出在上個月有否經歷有關情況。如有，請按程度評分。

					從未因此煩惱							非常煩惱
							0	1	2	3	4	5	6	
1	潮熱	不【 】	【 】是	→	0	1	2	3	4	5	6	
2	夜汗	不【 】	【 】是	→	0	1	2	3	4	5	6	
3	易汗	不【 】	【 】是	→	0	1	2	3	4	5	6	
4	對個人生活不滿	不【 】	【 】是	→	0	1	2	3	4	5	6	
5	煩躁易怒	不【 】	【 】是	→	0	1	2	3	4	5	6	
6	記憶力差	不【 】	【 】是	→	0	1	2	3	4	5	6	
7	工作能力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
8	抑鬱感	不【 】	【 】是	→	0	1	2	3	4	5	6	
9	不耐煩與人相處	不【 】	【 】是	→	0	1	2	3	4	5	6	
10	希望或需要獨處	不【 】	【 】是	→	0	1	2	3	4	5	6	
11	胃腸脹氣或脹氣疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
12	肌肉或關節疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
13	疲勞感	不【 】	【 】是	→	0	1	2	3	4	5	6	
14	難以入眠	不【 】	【 】是	→	0	1	2	3	4	5	6	
15	頭痛或頭痛	不【 】	【 】是	→	0	1	2	3	4	5	6	

MENQOL-1

更年期生活質素調查問卷(第 2次見面)

Page 2 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
☐☐☐	ICM/CTS/05/336	☐☐☐	☐☐ / ☐☐ / ☐☐ (DAY / MONTH / YEAR)

在下列各題中，請指出在上個月有否經歷有關情況。如有，請按程度評分。

					從未因此煩惱							非常煩惱
					0	1	2	3	4	5	6	
16	體力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
17	耐力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
18	精力匱乏	不【 】	【 】是	→	0	1	2	3	4	5	6	
19	皮膚乾燥	不【 】	【 】是	→	0	1	2	3	4	5	6	
20	體重增加	不【 】	【 】是	→	0	1	2	3	4	5	6	
21	面 毛增加	不【 】	【 】是	→	0	1	2	3	4	5	6	
22	皮膚外觀、質感和彈性改變	不【 】	【 】是	→	0	1	2	3	4	5	6	
23	感覺腸胃氣脹	不【 】	【 】是	→	0	1	2	3	4	5	6	
24	腰背疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
25	尿頻	不【 】	【 】是	→	0	1	2	3	4	5	6	
26	大笑或咳嗽時容易尿失禁	不【 】	【 】是	→	0	1	2	3	4	5	6	
27	性慾改變	不【 】	【 】是	→	0	1	2	3	4	5	6	
28	性交時陰道乾燥	不【 】	【 】是	→	0	1	2	3	4	5	6	
29	逃避親密行爲	不【 】	【 】是	→	0	1	2	3	4	5	6	

MENQOL-1

MENOPAUSE QUALITY OF LIFE RECORD(Visit 2)

Page 1 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
[ ][ ][ ]	ICM/CTS/05/336	[ ][ ][ ]	[ ][ ] / [ ][ ] / [ ][ ] (DAY / MONTH / YEAR)

FOR EACH OF THE FOLLOWING ITEMS, INDICATE WHETHER YOU HAVE EXPERIENCED THE PROBLEM IN THE **PAST MONTH.**
IF YOU HAVE, RATE HOW MUCH YOU HAVE BEEN **BOTHERED** BY THE PROBLEM

					NOT AT ALL BOTHERED							EXTREMELY BOTHERED
					0	1	2	3	4	5	6	
1	HOT FLUSHES OR FLASHES	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
2	NIGHT SWEATS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
3	SWEATING	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
4	BEING DISSATISFIED WITH MY PERSONAL LIFE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
5	FEELING ANXIOUS OR NERVOUS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
6	EXPERIENCING POOR MEMORY	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
7	ACCOMPLISHED LESS THAN I USED TO	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
8	FEELING DEPRESSED, DOWN, OR BLUE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
9	BEING IMPATIENT WITH OTHER PEOPLE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
10	FEELING OR WANTING TO BE ALONE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
11	FLATULENCE (WIND) OR GAS PAINS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
12	ACHING IN MUSCLES AND JOINTS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
13	FEELING TIRED OR WORN OUT	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
14	DIFFICULTY SLEEPING	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
15	ACHES IN BACK OF NECK OR HEAD	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	

MENQOL-1

MENOPAUSE QUALITY OF LIFE RECORD(Visit 2)

Page 2 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
[ ][ ][ ]	ICM/CTS/05/336	[ ][ ][ ]	[ ][ ] / [ ][ ] / [ ][ ] (DAY / MONTH / YEAR)

FOR EACH OF THE FOLLOWING ITEMS, INDICATE WHETHER YOU HAVE EXPERIENCED THE PROBLEM IN THE **PAST MONTH.**
IF YOU HAVE, RATE HOW MUCH YOU HAVE BEEN **BOTHERED** BY THE PROBLEM.

					NOT AT ALL BOTHERED						EXTREMELY BOTHERED
16	DECREASE IN PHYSICAL STRENGTH	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
17	DECREASE IN STAMINA	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
18	FEELING A LACK OF ENERGY	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
19	DRYING SKIN	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
20	WEIGHT GAIN	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
21	INCREASE IN FACIAL HAIR	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
22	CHANGES IN APPEARANCE, TEXTURE, OR TONE OF YOUR SKIN	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
23	FEELING BLOATED	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
24	LOW BACKACHE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
25	FREQUENT URINATION	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
26	INVOLUNTARY URINATION WHEN LAUGHING OR COUGHING	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
27	CHANGE IN YOUR SEXUAL DESIRE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
28	VAGINAL DRYNESS DURING INTERCOURSE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
29	AVOIDING INTIMACY	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6

MENQOL-1

DAILY RECORD - TO BE COMPLETED AT VISIT 1, VISIT 2 AND VISIT 3

TABLETS STARTED ON: DAY ___ - MONTH ___ - YEAR ___

DAILY RECORD STARTED ON: DAY ___ - MONTH ___ - YEAR ___

SUBJECT NUMBER: _____

SUBJECT INITIALS: _____

TABLET TAKEN: ↓

Day of Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Tablets Taken: Enter 0 or 1																												

Hot Flush Definitions:

Mild = Fleeting warm sensation, no sweating, does not disrupt activity

Moderate = Warm sensation, with sweating, does not disrupt activity

Severe = Hot sensation with sweating, disrupts activity

Enter "0" = none or actual number of hot flushes by severity below.

Day of Cycle		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Hot Flushes	Mild																												
	Moderate																												
	Severe																												

Enter "0" = no or "1" = yes for each occurrence of the postmenopausal symptoms listed below.

Day of Cycle	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Night Sweats																												
Insomnia																												
Feeling Bloated																												
Vaginal Dryness or Irritation																												
Vaginal Itching																												
Painful Intercourse																												
Less Interest in Intimacy																												
Painful urination or urgency																												
Palpitations																												
Malaise																												
Body or Joint Aches/Pains																												
Mood Swings																												
Decline in Memory																												
Loss of Concentration																												
Nervousness/Irritability																												
Skin Texture Changes																												
Hair Texture Changes																												

每日記錄卡

服用沖劑的首日日期:

□□ 日 · □□ 月 · □□ 年

Protocol Code
ICM/CTS/05/336

病人號碼: _____

病人姓名簡寫: _____ (V.)

_____月_____年

服藥	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
	已服用沖劑請填上「1」，如無則填上「0」																																
潮熱	*潮熱定義：* *輕微* = *感覺溫熱但不會出汗，並未影響起居生活* *中度* = *感覺溫熱會出汗，並未影響起居生活* *嚴重* = *感覺溫熱且出汗並影響起居生活*																																
	請依照上列嚴重程度填上當天潮熱次數，如無則填上「0」																																
	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
	潮熱： 輕微																																
	潮熱： 中度																																
	潮熱： 嚴重																																
夜汗	如出現夜汗情況請填上「1」，如無則填上「0」																																
	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
	夜汗																																

_____月_____年

服藥	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
	已服用沖劑請填上「1」，如無則填上「0」																																
潮熱	*潮熱定義：* *輕微* = *感覺溫熱但不會出汗，並未影響起居生活* *中度* = *感覺溫熱會出汗，並未影響起居生活* *嚴重* = *感覺溫熱且出汗並影響起居生活*																																
	請依照上列嚴重程度填上當天潮熱次數，如無則填上「0」																																
	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
	潮熱： 輕微																																
	潮熱： 中度																																
	潮熱： 嚴重																																
夜汗	如出現夜汗情況請填上「1」，如無則填上「0」																																
	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
	夜汗																																

每次覆診，請帶同此日記紙

Protocol Code: **ICM/CTS/004**

Subject Number: ☐☐☐☐

Subject Initials: ☐☐☐ (F M L)

CONCOMITANT MEDICATION-Visit 2 / Day 31

Please record all current and previous (within past 60 days) medications if tick Yes

Concomitant Medication ☐ 1 Yes ☐ 2 No

Drug name (Enter Generic name)	Indication (Use standard medical terminology)	**Start Date (if known) and Stopped Date** (DD-MM-YY)	Check if Continuing	Unit Dose (e g , 10mg, 10 units)	Frequency*	Route** (enter code)	Was drug administered for prophylaxis
1		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
2		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
3		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
4		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
5		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
6		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No

*Frequency 0=PRN 1=QD 2=BID 3=TID 4=QID 5=Other (specify)

**Route 1=IM 2=IV 3=PO 4=SC 5=Rectal 6=Topical 7=Nasal 8=Inhaled 9=Other (Specify)

Protocol Code	Subject Initials	Subject Number	Visit Date
ICM/CTS/05/336	F M L		D D M M Y Y

Visit 3 / Day 92

Vital Signs

Temperature (C^0) ☐☐.☐ Weight (kg) ☐☐☐.☐

Blood Pressure, Heart Rate and Body Mass Index

Time of measurement ☐☐ : ☐☐ (24 hour clock)

Measurement	Systolic (mm Hg)	Diastolic (mm Hg)
Blood Pressure	☐☐☐	☐☐☐

Heart Rate (beats / min)	☐☐☐
Body Mass Index (kg/m^2)	☐☐.☐

Laboratory Tests

Date of Blood Drawing ☐☐☐☐☐☐ (D D M M Y Y)

Protocol Code	Subject Initials	Subject Number	Visit Date
ICM/CTS/05/336	F M L		D D M M Y Y

Visit 3 / Day 92

Laboratory Tests

List below all laboratory findings

Attached the laboratory report to this page

Time Specimens Obtained ☐☐ : ☐☐ (24-hour clock)

		Result
Hematology	Hemoglobin	
	Erythrocytes	
	White Blood Count	
Biochemistry	Albumin	
	Calcium	
	Alkaline Phosphates	
	Phosphatase Inorganic	
	ALT/GPT	
	Bilirubin	
	Total Protein	
	Creatinine	
	Potassium	
	Sodium	
	Glucose	
	Urea	

		Result
Hormone	FSH	
	LH	
	E2	
Lipids	CHOL	
	LDL-C	
	HDL-C	
	TG	

Medication Accountability

No of capsules returned ☐☐ Missing dose ☐☐

Reason _____

停經後生活質素調查問卷(第 3次見面)

Page 1 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
	ICM/CTS/05/336		□□ / □□ / □□ DAY / MONTH / YEAR

在下列各題中，請指出在上個月有否經歷有關情況。如有，請按程度評分。

					從未因此煩惱							非常煩惱
					0	1	2	3	4	5	6	
1	潮熱	不【 】	【 】是	→	0	1	2	3	4	5	6	
2	夜汗	不【 】	【 】是	→	0	1	2	3	4	5	6	
3	易汗	不【 】	【 】是	→	0	1	2	3	4	5	6	
4	對個人生活不滿	不【 】	【 】是	→	0	1	2	3	4	5	6	
5	煩躁易怒	不【 】	【 】是	→	0	1	2	3	4	5	6	
6	記憶力差	不【 】	【 】是	→	0	1	2	3	4	5	6	
7	工作能力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
8	抑鬱感	不【 】	【 】是	→	0	1	2	3	4	5	6	
9	不耐煩與人相處	不【 】	【 】是	→	0	1	2	3	4	5	6	
10	希望或需要獨處	不【 】	【 】是	→	0	1	2	3	4	5	6	
11	胃腸脹氣或脹氣疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
12	肌肉或關節疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
13	疲勞感	不【 】	【 】是	→	0	1	2	3	4	5	6	
14	難以入眠	不【 】	【 】是	→	0	1	2	3	4	5	6	
15	頸痛或頭痛	不【 】	【 】是	→	0	1	2	3	4	5	6	

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更年期生活質素調查問卷(第 3次見面)

Page 2 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
☐☐☐	ICM/CTS/05/336	☐☐☐	☐☐ / ☐☐ / ☐☐ (DAY / MONTH / YEAR)

在下列各題中，請指出在上個月有否經歷有關情況。如有，請按程度評分。

					從未因此煩惱							非常煩惱
					0	1	2	3	4	5	6	
16	體力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
17	耐力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
18	精力匱乏	不【 】	【 】是	→	0	1	2	3	4	5	6	
19	皮膚乾燥	不【 】	【 】是	→	0	1	2	3	4	5	6	
20	體重增加	不【 】	【 】是	→	0	1	2	3	4	5	6	
21	面 毛增加	不【 】	【 】是	→	0	1	2	3	4	5	6	
22	皮膚外觀、質感和彈性改變	不【 】	【 】是	→	0	1	2	3	4	5	6	
23	感覺腸胃氣脹	不【 】	【 】是	→	0	1	2	3	4	5	6	
24	腰背疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
25	尿頻	不【 】	【 】是	→	0	1	2	3	4	5	6	
26	大笑或咳嗽時容易尿失禁	不【 】	【 】是	→	0	1	2	3	4	5	6	
27	性慾改變	不【 】	【 】是	→	0	1	2	3	4	5	6	
28	性交時陰道乾燥	不【 】	【 】是	→	0	1	2	3	4	5	6	
29	逃避親密行爲	不【 】	【 】是	→	0	1	2	3	4	5	6	

MENQOL-1

MENOPAUSE QUALITY OF LIFE RECORD(Visit 3)

Page 1 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
[ ][ ][ ]	ICM/CTS/05/336	[ ][ ][ ]	[ ][ ] / [ ][ ] / [ ][ ]
			DAY / MONTH / YEAR

FOR EACH OF THE FOLLOWING ITEMS, INDICATE WHETHER YOU HAVE EXPERIENCED THE PROBLEM IN THE **PAST MONTH.**
IF YOU HAVE, RATE HOW MUCH YOU HAVE BEEN **BOTHERED** BY THE PROBLEM

					NOT AT ALL BOTHERED							EXTREMELY BOTHERED
					0	1	2	3	4	5	6	
1	HOT FLUSHES OR FLASHES	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
2	NIGHT SWEATS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
3	SWEATING	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
4	BEING DISSATISFIED WITH MY PERSONAL LIFE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
5	FEELING ANXIOUS OR NERVOUS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
6	EXPERIENCING POOR MEMORY	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
7	ACCOMPLISHED LESS THAN I USED TO	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
8	FEELING DEPRESSED, DOWN, OR BLUE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
9	BEING IMPATIENT WITH OTHER PEOPLE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
10	FEELING OR WANTING TO BE ALONE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
11	FLATULENCE (WIND) OR GAS PAINS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
12	ACHING IN MUSCLES AND JOINTS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
13	FEELING TIRED OR WORN OUT	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
14	DIFFICULTY SLEEPING	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
15	ACHES IN BACK OF NECK OR HEAD	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	

MENQOL-1

MENOPAUSE QUALITY OF LIFE RECORD(Visit 3)

Page 2 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
	ICM/CTS/05/336		DAY / MONTH / YEAR

FOR EACH OF THE FOLLOWING ITEMS, INDICATE WHETHER YOU HAVE EXPERIENCED THE PROBLEM IN THE **PAST MONTH.**
IF YOU HAVE, RATE HOW MUCH YOU HAVE BEEN **BOTHERED** BY THE PROBLEM

					NOT AT ALL BOTHERED						EXTREMELY BOTHERED
16	DECREASE IN PHYSICAL STRENGTH	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
17	DECREASE IN STAMINA	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
18	FEELING A LACK OF ENERGY	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
19	DRYING SKIN	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
20	WEIGHT GAIN	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
21	INCREASE IN FACIAL HAIR	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
22	CHANGES IN APPEARANCE, TEXTURE, OR TONE OF YOUR SKIN	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
23	FEELING BLOATED	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
24	LOW BACKACHE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
25	FREQUENT URINATION	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
26	INVOLUNTARY URINATION WHEN LAUGHING OR COUGHING	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
27	CHANGE IN YOUR SEXUAL DESIRE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
28	VAGINAL DRYNESS DURING INTERCOURSE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
29	AVOIDING INTIMACY	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6

MENQOL-1

DAILY RECORD - TO BE COMPLETED AT VISIT 1, VISIT 2 AND VISIT 3

TABLETS STARTED ON: ☐☐ - ☐☐ - ☐☐ (DAY - MONTH - YEAR)

DAILY RECORD STARTED ON: ☐☐ - ☐☐ - ☐☐ (DAY - MONTH - YEAR)

SUBJECT NUMBER: _____

SUBJECT INITIALS: _____

TABLET TAKEN: ↓

Day of Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Tablets Taken. Enter 0 or 1																												

Hot Flush Definitions:

Mild = *Fleeting warm sensation, no sweating, does not disrupt activity*

Moderate = *Warm sensation, with sweating, does not disrupt activity*

Severe = *Hot sensation with sweating, disrupts activity*

Enter "0" = none or actual number of hot flushes by severity below.

Day of Cycle		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Hot Flushes	Mild																												
	Moderate																												
	Severe																												

Enter "0" = no or "1" = yes for each occurrence of the postmenopausal symptoms listed below.

Day of Cycle	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Night Sweats																												
Insomnia																												
Feeling Bloated																												
Vaginal Dryness or Irritation																												
Vaginal Itching																												
Painful Intercourse																												
Less Interest in Intimacy																												
Painful urination or urgency																												
Palpitations																												
Malaise																												
Body or Joint Aches/Pains																												
Mood Swings																												
Decline in Memory																												
Loss of Concentration																												
Nervousness/Irritability																												
Skin Texture Changes																												
Hair Texture Changes																												

每日記錄卡

服用沖劑的首日日期:

□□ - □□ - □□
日 月 年

Protocol Code
ICM/CTS/05/336

病人號碼: _____

病人姓名簡寫: _____ (V.)

_____月_____年

服藥	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	已服用沖劑請填上「1」，如無則填上「0」																															

潮熱定義： 輕微 = 感覺溫熱但不會出汗，並未影響起居生活
中度 = 感覺溫熱會出汗，並未影響起居生活
嚴重 = 感覺溫熱且出汗並影響起居生活

潮熱	請依照上列嚴重程度，填上當天潮熱次數，如無則填上「0」																															
	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	潮熱： 輕微																															
	潮熱： 中度																															
	潮熱： 嚴重																															

夜汗	如出現夜汗情況請填上「1」，如無則填上「0」																															
	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	夜汗																															

_____月_____年

服藥	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	已服用沖劑請填上「1」，如無則填上「0」																															

潮熱定義： 輕微 = 感覺溫熱但不會出汗，並未影響起居生活
中度 = 感覺溫熱會出汗，並未影響起居生活
嚴重 = 感覺溫熱且出汗並影響起居生活

潮熱	請依照上列嚴重程度，填上當天潮熱次數，如無則填上「0」																															
	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	潮熱： 輕微																															
	潮熱： 中度																															
	潮熱： 嚴重																															

夜汗	如出現夜汗情況請填上「1」，如無則填上「0」																															
	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	夜汗																															

每次覆診，請帶同此日記紙

Protocol Code: ICM/CTS/05/336

Subject Number: ____

Subject Initials: ___ (F M L)

CONCOMITANT MEDICATION-Visit 3 / Day 92

Please record all current and previous (within past 60 days) medications if tick Yes **Concomitant Medication** ☐ 1 Yes ☐ 2 No

Drug name (Enter Generic name)	Indication (Use standard medical terminology)	**Start Date (if known) and Stopped Date** (DD-MM-YY)	Check if Continuing	Unit Dose (e g , 10mg, 10 units)	Frequency*	Route** (enter code)	Was drug administered for prophylaxis
1		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
2		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
3		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
4		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
5		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
6		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No

*Frequency 0=PRN 1=QD 2=BID 3=TID 4=QID 5=Other (specify)

**Route 1=IM 2=IV 3=PO 4=SC 5=Rectal 6=Topical 7=Nasal 8=Inhaled 9=Other (Specify)

Protocol Code	Subject Initials	Subject Number	Visit Date
ICM/CTS/05/336	F M L		D D M M Y Y

Follow-up / Visit 4 / Day 120/Early Discontinuation

Premature termination of trial ☐ 1 yes ☐ 2 No

Reasons _____

Vital Signs

Temperature (C^0) ☐☐ . ☐ Weight (kg) ☐☐☐ . ☐

Blood Pressure, Heart Rate and Body Mass Index

Time of measurement ☐☐ : ☐☐ (24 hour clock)

Measurement	Systolic (mm Hg)	Diastolic (mm Hg)
Blood Pressure	☐☐☐ -	☐☐☐

Heart Rate (beats / min)	☐☐☐
Body Mass Index (kg/m^2)	☐☐ . ☐

停經後生活質素調查問卷

Page 1 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
	ICM/CTS/05/336		☐☐ / ☐☐ / ☐☐ DAY / MONTH / YEAR

在下列各題中，請指出在上個月有否經歷有關情況。如有，請按程度評分。

					從未因此煩惱							非常煩惱
					0	1	2	3	4	5	6	
1	潮熱	不【 】	【 】是	→	0	1	2	3	4	5	6	
2	夜汗	不【 】	【 】是	→	0	1	2	3	4	5	6	
3	易汗	不【 】	【 】是	→	0	1	2	3	4	5	6	
4	對個人生活不滿	不【 】	【 】是	→	0	1	2	3	4	5	6	
5	煩躁易怒	不【 】	【 】是	→	0	1	2	3	4	5	6	
6	記憶力差	不【 】	【 】是	→	0	1	2	3	4	5	6	
7	工作能力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
8	抑鬱感	不【 】	【 】是	→	0	1	2	3	4	5	6	
9	不耐煩與人相處	不【 】	【 】是	→	0	1	2	3	4	5	6	
10	希望或需要獨處	不【 】	【 】是	→	0	1	2	3	4	5	6	
11	胃腸脹氣或脹氣疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
12	肌肉或關節疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
13	疲勞感	不【 】	【 】是	→	0	1	2	3	4	5	6	
14	難以入眠	不【 】	【 】是	→	0	1	2	3	4	5	6	
15	頭痛或頭痛	不【 】	【 】是	→	0	1	2	3	4	5	6	

MENQOL-1

更年期生活質素調查問卷

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
	ICM/CTS/05/336		☐☐ / ☐☐ / ☐☐ DAY / MONTH / YEAR

在下列各題中，請指出在上個月有否經歷有關情況。如有，請按程度評分。

					從未因此煩惱							非常煩惱
					0	1	2	3	4	5	6	
16	體力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
17	耐力下降	不【 】	【 】是	→	0	1	2	3	4	5	6	
18	精力匱乏	不【 】	【 】是	→	0	1	2	3	4	5	6	
19	皮膚乾燥	不【 】	【 】是	→	0	1	2	3	4	5	6	
20	體重增加	不【 】	【 】是	→	0	1	2	3	4	5	6	
21	面 毛增加	不【 】	【 】是	→	0	1	2	3	4	5	6	
22	皮膚外觀、質感和彈性改變	不【 】	【 】是	→	0	1	2	3	4	5	6	
23	感覺腸胃氣脹	不【 】	【 】是	→	0	1	2	3	4	5	6	
24	腰背疼痛	不【 】	【 】是	→	0	1	2	3	4	5	6	
25	尿頻	不【 】	【 】是	→	0	1	2	3	4	5	6	
26	大笑或咳嗽時容易尿失禁	不【 】	【 】是	→	0	1	2	3	4	5	6	
27	性慾改變	不【 】	【 】是	→	0	1	2	3	4	5	6	
28	性交時陰道乾燥	不【 】	【 】是	→	0	1	2	3	4	5	6	
29	逃避親密行爲	不【 】	【 】是	→	0	1	2	3	4	5	6	

MENQOL-1

MENOPAUSE QUALITY OF LIFE RECORD

Page 1 of 2

SUBJECTT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
	ICM/CTS/05/336		DAY / MONTH / YEAR

FOR EACH OF THE FOLLOWING ITEMS, INDICATE WHETHER YOU HAVE EXPERIENCED THE PROBLEM IN THE **PAST MONTH.**
IF YOU HAVE, RATE HOW MUCH YOU HAVE BEEN **BOTHERED** BY THE PROBLEM.

					NOT AT ALL BOTHERED							EXTREMELY BOTHERED
					0	1	2	3	4	5	6	
1	HOT FLUSHES OR FLASHES	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
2	NIGHT SWEATS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
3	SWEATING	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
4	BEING DISSATISFIED WITH MY PERSONAL LIFE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
5	FEELING ANXIOUS OR NERVOUS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
6	EXPERIENCING POOR MEMORY	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
7	ACCOMPLISHED LESS THAN I USED TO	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
8	FEELING DEPRESSED, DOWN, OR BLUE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
9	BEING IMPATIENT WITH OTHER PEOPLE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
10	FEELING OR WANTING TO BE ALONE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
11	FLATULENCE (WIND) OR GAS PAINS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
12	ACHING IN MUSCLES AND JOINTS	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
13	FEELING TIRED OR WORN OUT	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
14	DIFFICULTY SLEEPING	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	
15	ACHES IN BACK OF NECK OR HEAD	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6	

MENQOL-1

MENOPAUSE QUALITY OF LIFE RECORD

Page 2 of 2

SUBJECT NO.	Protocol Code	SUBJECT INITIALS	DATE OF VISIT
[ ][ ][ ]	ICM/CTS/05/336	[ ][ ][ ]	[ ][ ] / [ ][ ] / [ ][ ] DAY / MONTH / YEAR

FOR EACH OF THE FOLLOWING ITEMS, INDICATE WHETHER YOU HAVE EXPERIENCED THE PROBLEM IN THE **PAST MONTH.**
IF YOU HAVE, RATE HOW MUCH YOU HAVE BEEN **BOTHERED** BY THE PROBLEM

					NOT AT ALL BOTHERED						EXTREMELY BOTHERED
16	DECREASE IN PHYSICAL STRENGTH	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
17	DECREASE IN STAMINA	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
18	FEELING A LACK OF ENERGY	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
19	DRYING SKIN	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
20	WEIGHT GAIN	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
21	INCREASE IN FACIAL HAIR	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
22	CHANGES IN APPEARANCE, TEXTURE, OR TONE OF YOUR SKIN	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
23	FEELING BLOATED	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
24	LOW BACKACHE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
25	FREQUENT URINATION	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
26	INVOLUNTARY URINATION WHEN LAUGHING OR COUGHING	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
27	CHANGE IN YOUR SEXUAL DESIRE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
28	VAGINAL DRYNESS DURING INTERCOURSE	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6
29	AVOIDING INTIMACY	NO [ ]	[ ] YES	→	0	1	2	3	4	5	6

MENQOL-1

DAILY RECORD - TO BE COMPLETED AT VISIT 1, VISIT 2 AND VISIT 3

TABLETS STARTED ON: ___ ___ - ___ ___ - ___ ___ (DAY - MONTH - YEAR)

DAILY RECORD STARTED ON: ___ ___ - ___ ___ - ___ ___ (DAY - MONTH - YEAR)

SUBJECT NUMBER: _____

SUBJECT INITIALS: _____

TABLET TAKEN: ↓

Day of Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Tablets Taken: Enter 0 or 1																												

Hot Flush Definiations:

Mild = *Fleeting warm sensation, no sweating, does not disrupt activity*

Moderate = *Warm sensation, with sweating, does not disrupt activity*

Severe = *Hot sensation with sweating, disrupts activity*

Enter "0" = none or actual number of hot flushes by severity below.

Day of Cycle		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Hot Flushes	Mild																												
	Moderate																												
	Severe																												

Enter "0" = no or "1" = yes for each occurrence of the postmenopausal symptoms listed below.

Day of Cycle	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Night Sweats																												
Insomnia																												
Feeling Bloated																												
Vaginal Dryness or Irritation																												
Vaginal Itching																												
Painful Intercourse																												
Less Interest in Intimacy																												
Painful urination or urgency																												
Palpitations																												
Malaise																												
Body or Joint Aches/Pains																												
Mood Swings																												
Decline in Memory																												
Loss of Concentration																												
Nervousness/Irritability																												
Skin Texture Changes																												
Hair Texture Changes																												

每日記錄卡

服用沖劑的首日日期:

□□ - □□ - □□
日 月 年

Protocol Code
ICM/CTS/05/336

病人號碼: _____

病人姓名簡寫: _____ (V.)

_____月_____年

服藥	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31		
	已服用沖劑請填上「1」，如無則填上「0」																																	
潮熱	*潮熱定義：* *輕微* = *感覺溫熱但不會出汗，並未影響起居生活* *中度* = *感覺溫熱會出汗，並未影響起居生活* *嚴重* = *感覺溫熱且出汗並影響起居生活*																																	
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	夜汗																																	

_____月_____年

服藥	日期	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31		
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	夜汗																																	

每次覆診，請帶同此日記紙

Protocol Code: ICM/CTS/05/336

Subject Number: ☐☐☐☐

Subject Initials: ☐☐☐ (F M L)

CONCOMITANT MEDICATION-Follow-up / Visit 4 / Day 120

Please record all current and previous (within past 60 days) medications if tick Yes

Concomitant Medication ☐ 1 Yes ☐ 2 No

Drug name (Enter Generic name)	Indication (Use standard medical terminology)	**Start Date (if known) and Stopped Date** (DD-MM-YY)	Check if Continuing	Unit Dose (e g , 10mg, 10 units)	Frequency*	Route** (enter code)	Was drug administered for prophylaxis
1		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
2		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
3		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
4		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
5		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No
6		Start Date ☐☐☐☐☐☐ Stop Date ☐☐☐☐☐☐	☐ 1C				☐ 1 Yes ☐ 2 No

*Frequency 0=PRN 1=QD 2=BID 3=TID 4=QID 5=Other (specify)

**Route 1=IM 2=IV 3=PO 4=SC 5=Rectal 6=Topical 7=Nasal 8=Inhaled 9=Other (Specify)

Menopausal Symptoms – Greene Climacteric Scale

Screening, Baseline, Visit 2, Visit 3 and Follow-up

Have you ever experienced any of the following in the past 1 month?

	None	Slight	Moderate	Marked
1. Heart beating quickly or strongly				
2. Feeling tense or nervous				
3. Difficulty in sleeping				
4. Excitable				
5. Attacks of panic				
6. Difficulty in concentrating				
7. Feeling tired or lacking in energy				
8. Loss of interest in most things				
9. Feeling unhappy or depressed				
10.Crying spells				
11.Irritability				
12.Feeling dizzy or faint				
13.Pressure or tightness in head or body				
14.Parts of body feel numb or tingling				
15.Headaches				
16.Muscle and joint pains				
17.Loss of feeling in hands or feet				
18.Breathing difficulties				
19.Hot flushes				
20.Sweating at night				
21.Loss of interest in sex				
22.Increased urinary frequency				
23.Urinary incontinence				
24.Vaginal dryness				

絕經症狀

在過去的一個月內您有否感 受到以下的症狀問題?

	沒有	輕 微	中等	嚴重
1. 急速或強力心跳	□	□	□	□
2. 感到緊張	□	□	□	□
3. 失眠	□	□	□	□
4. 興奮	□	□	□	□
5. 突然感到驚慌	□	□	□	□
6. 難以集中精神	□	□	□	□
7. 感到疲倦或缺乏精力	□	□	□	□
8. 對大部份事物缺乏興趣	□	□	□	□
9. 感到不開心或憂愁	□	□	□	□
10.哭泣	□	□	□	□
11.急躁	□	□	□	□
12.感到頭暈/暈眩	□	□	□	□
13.頭部或身體感到有	□	□	□	□
14.身體部份感到麻痺或刺痛	□	□	□	□
15.頭痛	□	□	□	□
16.肌肉與關節疼痛	□	□	□	□
17.手腳失去感覺	□	□	□	□
18.呼吸困難	□	□	□	□
19.潮熱	□	□	□	□
20.夜汗	□	□	□	□
21.對性事失去興趣	□	□	□	□
22.頻尿	□	□	□	□
23.小便失禁	□	□	□	□
24.陰道乾涸	□	□	□	□

Protocol Code: **ICM/CTS/05/336**

Subject Initials: F M L

Subject Number

NON-SERIOUS ADVERSE EVNETS (MINIMUM - ALL COLUMNS REQUIRED) - Day ___

Did the subject experience any non-serious adverse event during the study? Yes [ ]1 Y No [ ]2 N If YES, indicate below

Non-serious adverse events	Date of Onset	Maximum Intensity	Outcome	Date of Resolution	Action taken with study medicaiton as a result of this non-serious AE?	Withdrawal	Relationship to study medication	Seriousness
Diagnosis only (if known) or signs/symptoms *(list one per line)*	day month year	1=Mild 2=Moderate 3=Severe 4=Not applicable	R=Resolved S=Resolved with sequelae N=Not resolved	day month year	0=None 1=Dosage adjusted 2=Temporarily interrupted 3=Permanently discontinued 4=Not applicable	Did subject withdraw from study as a result of this non-serious AE? Y=Yes N=No	Is there a reasonable possibility that the non-serious AE may have been caused by the study med est on? Y=Yes N=No	Does the AE meet the definition of serious? Y=Yes N=No
e g *RASH*	*20 AUG 96*	*3*	*R*	*22 AUG 96*	*0*	*N*	*Y*	*N*
1								N
2								N
3								N
4								N
5								N
6								N
7								N

Standard Serious Adverse Event Form

Jan/2005

Serious Adverse Event Report Tel 2632-3935 Fax 2646-6653 Website http //intranet ccter cuhk edu hk/sae/	Protocol code (CRE#)	Type of report ☐ Initial ☐ Follow-up (case not completed) ☐ Follow-up (case completed)	Date of this report day / month / year

Patient Identification

Patient No	Patient Initials			Date of birth			Sex	Date of enrolment		
	first	mid	last	day	month	year	☐ Male ☐ Female	day	month	year

Serious Adverse Event

Onset of event			End of event		
day	month	year	day	month	year

Tick all appropriate to serious adverse event

- ☐ patient died, date of death day month year
  - Autopsy
    - ☐ Yes
    - ☐ Planned
    - ☐ No
- ☐ life threatening
- ☐ required inpatient hospitalization or prolongation of existing hospitalization
- ☐ persistent or significant disability/incapacity
- ☐ congenital anomaly/birth defect
- ☐ other (specify) _____

Description of SAE (use extra paper if necessary, enclose a discharge summary if any)

1 Diagnosis/syndrome _____

2 Narrative description of SAE

Outcome of Event to Date

- ☐ complete recovery
- ☐ recovery with sequelae
- ☐ present at time of this report
- ☐ unknown
- ☐ died

If the patient died, cause of death _____

Causal relationship between death and study drug/treatment

- ☐ associated
- ☐ possible associated
- ☐ not associated --give alternative explanation _____

Study Drug/treatment Information

Study drug/treatment	Dose, frequency & route used (if applicable)	Indication	Therapy dates [day/month/year] from	Therapy dates [day/month/year] to

Causal relationship between study drug/treatment and event

- ☐ associated
- ☐ possible associated
- ☐ not associated -give alternative explanation
- ☐ unknown

Action taken with study drug / treatment as a result of the SAE

- ☐ None
- ☐ Dosage adjusted
- ☐ Temporarily interrupted
- ☐ Permanently discontinued

Withdrawn from study as a result of the SAE

- ☐ Yes
- ☐ No

Concomitant Drug/treatment and Relevant Medical History

Relevant medical history [e g previous diagnoses, surgery, allergies, pregnancy with date of last period (day/month/year), use extra paper if necessary]

Concomitant drug/treatment (exclude those used to treat the SAE, use extra paper if necessary)

Drug/treatment	Dose, frequency & route used (if applicable)	Indication	Therapy dates [day/month/year] from	Therapy dates [day/month/year] to	Causal relationship to event
					☐ associated ☐ possible associated ☐ not associated
					☐ associated ☐ possible associated ☐ not associated
					☐ associated ☐ possible associated ☐ not associated
					☐ associated ☐ possible associated ☐ not associated

Investigator

Name _____ Signature _____ Tel _____ Fax _____

Appendix 5

PROSPECTIVE TRIAL OF A HERBAL FORMULA BYSH (舒尿靈) AND SAW PALMETTO IN PATIENTS WITH HORMONAL REFRACTORY PROSTATE CANCER- INVESTIGATOR BROCHURE

Sponsor:	Institute of Chinese Medicine, CUHK
Study Product:	BYSH (舒尿靈) and Saw Palmetto
Protocol Number:	ICM/CTS/05/335
Principle Investigator:	Dr. Ng Chi Fai
Investigator:	Professor Leung Ping Chung

Date: June 1, 2007

Amended:

Administrative Change:

Appendix 5

Table of Content

Appendix 5

1 Composition of the herbal formula

白花蛇舌草	Herba Hedyotis diffusae
薏苡仁	Semen Coicis
黄芩	Radix Scutellariae
三七	Radix Notoginseng
棕榈子	saw palmetto

白花蛇舌草 Herba Hedytidis diffusae

The herb contains oleanolic acid, -sitosterol, para-coumaric acid, flavonoid glycoside, oldenlandin, etc

薏苡仁 Semen Coicis

Semen Coicis contains about 52% starch, 18% protein, 7% fat, and coix oil, coixenolide, fatty oil, amino acids, sugar, etc

黄芩 Radix Scutellariae

The decoction of the herb has broad spectral antibacterial effect against pathogenic bacteria, such as Salmonella typhi, Shigella dysenteriae, staphylococci, etc

三七 Radix Notoginseng

This herb contains pseudoginseng saponin, flavonoid glycosides, quercetin, quercitrin, beta-sitosterol and B-N-Z-diacetyl-L-A,B-diaminopropionic acid, an active hemostatic ingredient

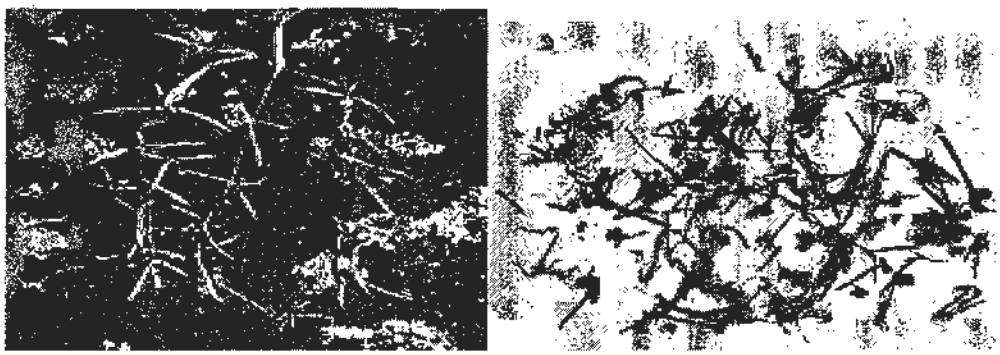
棕榈子 saw palmetto

The berries of saw palmetto contain approximately 1 5% volatile oil, of which 63% are free fatty acids and 38% are ethyl esters of those fatty acids The fatty acids include caproic, caprylic, capric, lauric, palmitic, and oleic acids, and ethyl esters of these In addition, the berries contain beta-sitosterol and its glucoside, beta sitosterol D-glucoside, as well as ferulic acids

Appendix 5

2 Information on the herbs used for the drug formula

2.1 白花蛇舌草 Herba Hedytidis diffusae



Origin:

The whole herb of Oldenlandia diffusa (Willd.), or Hedyotis diffusa (Willd.), an annual herb, of the Rubiaceae family. Native to East Asia, the plant is grown in China, Japan, Korea, Taiwan, India, Malaysia, the Philippines and Singapore.

The annual plant prefers moist ground and fields, it grows to 0.3 m by 0.3 m. It is in flower from August to September. The flowers are hermaphrodite (have both male and female organs). It can grow in semi-shade (light woodland).

In China, this herb is grown in the provinces to the south of the Yangtze River. It is harvested in summer and autumn. Used by washing it clean, drying in the sun and cutting into sections.

Properties:

Slightly bitter and sweet in flavor, cold in nature, it is related to the stomach, large intestine and small intestine channels.

Functions:

Clears away heat to expel toxic substances and induces diuresis to relieve strangury (painful discharge of urine).

Snake-needle grass is a pleasant-tasting cooling, alterative herb that lowers fever, reduces inflammation, relieves pain and is diuretic, depurative (purifying the blood), sedative and antibacterial. It acts mainly on the liver and also stimulates the immune system.

Applications:

1. For treating skin and external diseases, sore throat and snake bite:

(A) Skin and external diseases:

This herb can be used alone for oral ingestion and external application and it can also be used together with honeysuckle flower (Flos Lonicerae), weeping forsythia fruit (Fructus Forsythiae), mother chrysanthemum (Flos Chrysanthemi Indici), etc

(B) Abdominal pain.

Use it with sargent gloryvine stem (Caulis Sargentodoxae), patrinia (Herba Patriniae), tree peony root-bark, etc

(C) Sore throat

Use it with such herbs as skullcap root (Radix Scutellariae), Zhejiang figwort root (Radix Scrophulariae), dyers woad root (Radix Isatidis), etc

(D) Snake bite

This herb can be used alone or in combination with such herbs as sunplant (Herba Scutellariae Barbatae), Chinese violet (Herba Violae), manyleaf paris rhizome (Rhizoma Paridis Polyphyllae), etc

2 For treating strangury due to heat

Use it with sunplant (Herba Scutellariae Barbatae), Asiatic plantain (Herba Plantaginis), pyrrosia leaf (Folium Pyrrosiae), etc

Dosage and Administration:

15-60 g

Decoct the herb and other ingredients for drinking Use an adequate amount externally

Cautions on Use:

This herb should be avoided by anyone who suffers from deep-rooted carbuncles or who has any spleen-*yang* insufficiency

Reference Materials:

'Records of Guangxi Materia Medica'

"Oral ingestion for the treatment of malnutrition in children, snake bite and cancers and tumors and external use for the treatment of tinea and herpes zoster complicated by infection "

Modern Researches:

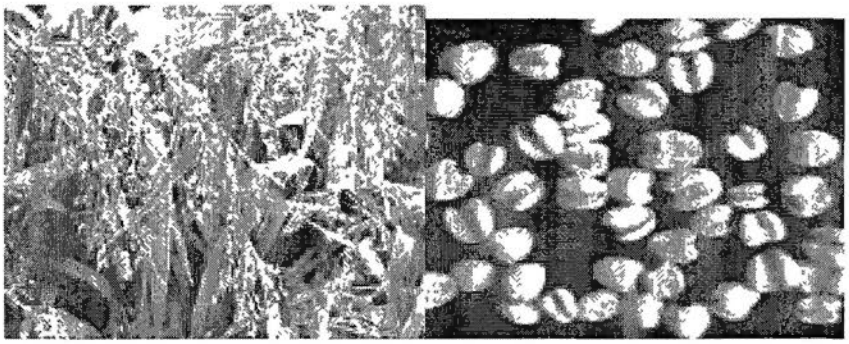
The herb contains oleanolic acid, -sitosterol, para-coumaric acid, flavonoid glycoside, oldenlandin, etc

In in vitro experiments, snake-needle grass shows no obvious antimicrobial effect, but it can stimulate the proliferation of the reticuloendothelial system to promote the formation of antibodies and strengthen the phagocytic power of reticular cells and white cells so as to achieve the aims of resisting bacteria and relieving inflammation

Snake-needle grass has an antineoplastic effect The injection of it into the abdominal cavities of animals can give tranquilizing, analgesic and hypnotic effects It can inhibit spermatogenic power and has liver-protective and cholagogic effects

Appendix 5

2.2 薏苡仁 Semen Coicis:



Origin:

The ripe seed of Coix lacrymajobi L., a perennial herb, of the Gramineae family.

The plant is a leafy, jointed-stemmed annual grass, native to tropical Asia and naturalized in North America, grown in wet places in grassland or in the foothills. It is 1 to 3 m tall. It is in leaf from May to October, in flower from July to October, and the seeds ripen from September to November. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by wind. The plant cannot grow in the shade. It requires moist soil.

Job's tears receives its name from the hard, shiny, tear-shaped beads that enclose the seed kernels. They are off-white or dark in colour and are 6 to 12 mm long. They are sometimes used for jewelry and rosaries. Job's tears is native to the Indian subcontinent but is now widespread throughout the tropical zone. It grows in marshy places and is cultivated in China, the seed kernel having a long-applied medicinal value. The seed kernel is also edible, and forms of it are used as cereal foods in parts of East Asia and in the Philippines.

In China, it is produced in most areas, but primarily in the provinces Fujian, Hebei, Liaoning, etc. Harvested in autumn when the fruit ripens, the plant is cut off, dried in the sun, the fruits beaten off, dried again in the sun, with the shells and seed skins removed for use when raw or after being parched.

Properties:

Sweet and bland in flavor, slightly cold in nature, it is related to the spleen, stomach and lung channels.

Functions:

Induces diuresis (increased excretion of urine), removes dampness and edema (excess accumulation of serous fluid in connective tissue), strengthens the spleen, eliminates stagnation, nourishes the digestive functions, clears away heat and promotes the discharge of pus.

In addition, the seeds can be ground into a flour and used to make bread or used in any of the ways that rice is used.

A tea can be made from the parched seeds.

A coffee is made from the roasted seeds.

Applications:

1. To treat dysuria (difficult or painful discharge of urine), edema (abnormal infiltration and excess accumulation of serous fluid in connective tissue), beriberi (inflammatory or degenerative changes of the nerves, digestive system, and heart) and diarrhea due to deficiency of the spleen:

a) Abdominal distention with edema, diarrhea with poor appetite, beriberi with edema of the feet, etc., due to deficiency of the spleen and exuberance of dampness:

This herb is mostly used in combination with such herbs as tuckahoe (Poria Cocos), largehead atractylodes rhizome (Rhizoma Atractylodis Macrocephalae), milk vetch root (Radix Astragali seu Hedysari), etc.

b) Strangury (painful discharge of urine) due to damp-heat pathogen:

Being rather cool in nature, this herb can clear away damp-heat, so it can also be used for strangury due to damp-heat pathogens. For example, Job's tears is decocted alone in the book 'Yang's Proven Recipes' for oral administration in order to treat strangury resulting from heat and urinary stones.

2. To treat damp arthralgia and spasms:

a) Rheumatism and handicapped movements of the limbs:

This herb is often used together with ephedra, apricot kernels and licorice, e.g., Ma Xing Yi Gan Tang.

Or, boil 30 g Job's tears and 30-60 g rice to make thick porridge. Consume once daily.

b) Prolonged arthralgia due to wind-dampness, spasms of the muscles and edema:

Job's tears are made into congee for oral administration, e.g., Yiyiren Zhou, in the book 'Dietitian's Experience'.

c) Such ailments due to stagnation of dampness and steaming heat accumulated in the channels and collaterals:

This herb can be used together with talcum and weeping forsythia fruit (Fructus Gardeniae), e.g., Xuanbi Tang.

d) Pain in lower limbs with swelling, also for hemoptysis (spitting of blood) or bloody sputum:

Use 50 g Job's tears and 2 pig's trotters. Simmer with slow fire and consume the whole thing.

3. To treat pulmonary abscess (pus in the lung) and acute appendicitis (inflammation of the vermiform appendix):

a) Pulmonary abscess with chest pain and sputum with mucus:

This herb is often used together with reed stem, Chinese waxgourd seed, peach kernels, etc., e.g., Weijing Tang in the book 'The Thousand Gold Remedies'.

b) Acute appendicitis

This herb can be used in combination with monkshood root (Radix Aconiti Praeparata), patrinia and tree peony root-bark, e g , Fuzi Yiyi Baijiang San

4 To treat warts

a) Reported in 'Eating Your Way to Health' A group of 23 cases were treated by Job's tears porridge by boiling 50 g Job's tears plus 50 g rice together This was administered once daily After 7-16 days, 11 cases saw their lesions subside, and 6 cases were ineffective

At the beginning of the therapy,the lesions enlarged and reddened However, persistent therapy for several more days resulted in the peeling off and withering of the lesions, which eventually dried and dropped off

b) Boil 40 g Job's tears for 20 minutes Divide into 2 equal halves and consume twice, in the morning and in the evening Ten successive days constitute a therapeutic course

Dosage and Administration:

10-30 g

Decoct this herb for oral administration It should be used when raw to clear away damp-heat, while it should be used after being parched to strengthen the spleen and arrest diarrhea Being slow in potency, this herb should be used in a large dose

In addition to its inclusion in a decoction, pills or powder, it can be made into congee for eating, which is a good foodstuff for dietetic therapy

Reference Materials:

'Shen Nong's Herbal Classic'

"To treat muscles spasms, inability of joints to bend and stretch, rheumatism and arthralgia by sending down adversely rising *qi* "

'The Compendium of Materia Medica'

"Being a herb for the *yangming* channel, Job's tears can strengthen the spleen and reinforce the stomach As it replenishes the organ with deficiency, it is used for consumptive lung diseases and pulmonary abscess As any disease of bones or muscles must basically be treated through the *yangming* channel, this herb is used for anyone suffering from spasms of muscles or migratory arthralgia As its earth nature can overcome water and eliminate dampness, it is used for diarrhea or edema "

Modern Researches:

Job's tears contains about 52% starch, 18% protein, 7% fat, and coix oil, coixenolide, fatty oil, amino acids, sugar, etc

It is higher in protein and fat than rice but low in minerals This is a potentially very useful grain It has a higher protein to carbohydrate ratio than any other cereal, though the hard seedcoat makes

extraction of the flour rather difficult.

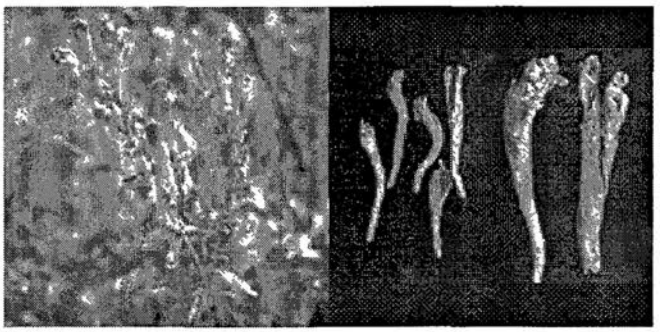
The coix oil can stop or reduce the contracture of striated muscles and stimulate the uterus. The oil can also reduce serum calcium and blood sugar, is antagonistic to caffeine, i.e., inhibition to the central nervous system, and it also has antipyretic (reduces fever), tranquilizing and analgesic (insensitive to pain) effects.

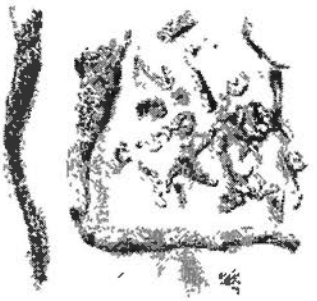
Its decoction has a certain inhibitory effect on cancer cells.

The roots have been used in the treatment of menstrual disorder.

Appendix 5

2.3 黄芩 Radix Scutellariae





Origin:

The root of Scutellaria Baicalensis Georgi, a perennial herb, of the Lamiaceae family. Native to the shores of Lake Baikal, Mongolia, Siberia, Hebei, Shanxi, Henan and Shantung provinces of China. The plants are now grown in North America and Europe.

The flowers of skullcap are mostly purple in color. The part used in traditional Chinese medicine is the dried root, which attains harvestable size after about two years.

Cultivation of the flowers can be easily done in a greenhouse. Seeds are sowed in early spring, and will be germinated in about 24 days. Transplant seedlings 30 cm apart. Grows to 30 cm tall. Prefers well-drained soil in the full sun. As the plants age they become wider, much like humans in middle age, but unlike humans, the seed they produce becomes increasingly viable the older they get.

The roots are reaped in spring and autumn. Fully steamed or fully moistened with boiled water, sliced and used when raw or after being stir-fried with wine or charred.

Properties:

Bitter in flavor, cold in nature, it is related to the lung, stomach, gall-bladder and large intestine channels.

Functions:

Clears away heat to eliminate dampness, purges intense heat to remove toxic substances, removes heat from the blood to stop bleeding and removing internal heat to prevent miscarriage.

Applications:

1. For treating damp-heat and summer-heat syndromes, feeling of fullness in the chest and upper abdomen due to damp-heat, jaundice and diarrhea:

(A) Damp-heat and summer-heat syndromes, warm obstruction due to damp-heat, feeling of fullness in the chest and upper abdomen, nausea with vomiting, recessive fever with yellow and greasy tongue fur:

It is used in combination with talcum, round cardamon seed (Semen Amomi Cardamomi), rice-paper plant stem pith (Medulla Tetrapanacis), etc., e.g., Huangqin Huashi Tang.

(B) Retention of damp-heat in the spleen and stomach, feeling of fullness in the chest and upper abdomen with vomiting:

It is used in combination with Chinese goldthread rhizome (Rhizoma Coptidis), dried ginger and pinellia tuber (Rhizoma Pinelliae), etc., e.g., Banxia Xiexin Tang, as a pungent preparation for dispersion and a bitter one for purgation.

(C) Diarrhea or dysentery due to damp-heat in the large intestine:

It is used in combination with Chinese goldthread rhizome (Rhizoma Coptidis) and kudzu vine root (Radix Puerariae), e.g., Gegen Qin Lian Tang.

(D) Jaundice due to damp-heat:

It is used in combination with Capillary artemisia (Herba Artemisiae Scopariae) and Gardenia fruit (Fructus Gardeniae).

2. For treating cough due to lung-heat and febrile diseases with excessive heat:

(A) Obstruction of the lung-heat, impairment of purifying and descending functions of the lungs and coughing with thick sputum:

This herb can be used to produce effects by itself, e.g., Qing Jin Wan, and together with white mulberry root-bark (Cortex Mori Radicis), windweed rhizome (Rhizoma Anemarrhenae), ophiopogon root (Radix Ophiopogonis), etc., e.g., Qing Fei Tang, in order to enhance the effects of removing heat from the lungs to relieve coughing.

(B) Febrile diseases due to exopathogens, high fever with excessive thirst caused by stagnation of heat in the chest and the spleen and stomach, flushed face with dried lips, reddish urine with constipation, yellow tongue fur with taut and rapid pulse:

It can be used in combination with Peppermint, Gardenia fruit (Fructus Gardeniae), rhubarb root (Radix et Rhizoma Rhei), etc., e.g., Liang Ge San, in order to purge intense heat to relieve constipation.

(C) Alternate attacks of chills and fever due to pathogenic factors in the *shaoyang* channel:

It can be used in combination with Chinese thorowax root (Radix Bupleuri), e.g., Xiao Chaihu Tang, and is effective in treating pathogens in *shaoyang* diseases with alternating chills and fever and in relieving the *shaoyang* channels.

3. For treating skin and external diseases and sore throat:

The herb can be used with honeysuckle flower (Flos Lonicerae), weeping forsythia fruit (Fructus Forsythiae), great burdock achene (Fructus Arctii), etc.

4. For treating epistaxis (nosebleed) and hematemesis (vomiting blood) due to blood-heat:

It can be used in combination with raw rehmannia, cogongrass rhizome (Rhizoma Imperatae), pseudo-ginseng root (Radix Onto-ginseng), etc.

5. For treating fetal irritability due to heat syndromes in pregnancy:
This herb can remove intense heat to prevent miscarriage.

Dosage and Administration:

3-10 g.

Decoct the ingredients for drinking. It is mostly used when raw to clear away heat, after being stir-fried to prevent miscarriage, charred to stop bleeding and fried in wine to remove heat from the chest.

This herb is also divided into the perennial root, which is good at clearing fire from the lungs; and the young root, which is good at clearing fire from the large intestine and purging damp-heat from the abdomen.

Cautions on Use:

This herb should be avoided by those with spleen-*yang* insufficiency.

Reference Materials:

'Shen Nong's Herbal Classic' :

"Primarily for jaundice due to all heat, spouting bleeding from the anus, diarrhea or dysentery, amenorrhea (abnormal absence or suppression of menses) due to deficiency of blood, malignant boils, carbuncles with ulcers and acute scleritis (inflammation of the sclera, the tough white outer coat of the eyeball)."

'The Pearl Bag (Zhenzhu Nang)' :

"Removing heat from the heart, eliminating damp-heat from the lungs, purging abnormally risen lung-fire, ... preventing miscarriage."

'Original Materia Medica' :

"The perennial root is indicated for dyspnea (difficult respiration) with coughs, bleeding, alternate attacks of chills and fever, wind-heat and damp-heat syndromes, wind syndromes of the head, infectious epidemic diseases, sore throat, consumptive lung diseases, acute mastitis, lumbodorsal carbuncles, skin eruptions, maculae and papulae, scrofula, skin and external diseases and acute conjunctivitis. The young root is indicated for dysentery with bloody stools, accumulation of heat in the bladder, five types of strangury (including uropsammus, urinary incontinence, galacturia, pollakiuria and hematuria), constipation, hemafecia and metrostaxis."

Modern Researches:

The decoction of the herb has broad spectral antibacterial effect against pathogenic bacteria, such as Salmonella typhi, Shigella dysenteriae, staphylococci, etc. It also possesses antipyretic, hypotensive, diuretic, sedative and cholagogic effects, and it can decrease the capillary permeability and inhibit peristalsis as well.

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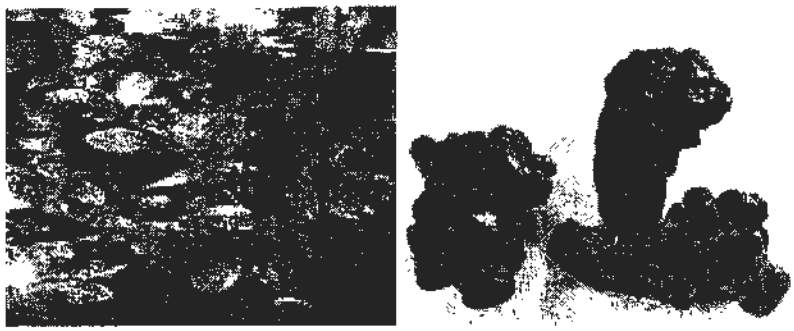
It functions similar to Chinese Goldthread Rhizome, but shows no presence of berberine or hydrastine.

2.4 三七 Radix Notoginseng



Origin:

The root of the perennial plant Panax pseudo-ginseng notoginseng (Burkill.) G.Hoo. & C.J.Tseng., of the family Araliaceae. Native to east Asia, it is found in forests and shrubberies in China, Burma, and up to 2100-4300 metres in Central Nepal and the Himalayas.

The perennial plant grows to about 1.2 m high at a slow rate. The flowers are hermaphrodite (have both male and female organs). It can grow in full shade (deep woodland) or semi-shade (light woodland). It requires moist soil.

In China, notoginseng is mainly grown in the provinces Yunnan, Guangxi, etc. The root is harvested in autumn before flowering or after the seed has ripened, then dried in the sun for use when raw.

Properties:

Sweet and slightly bitter in flavor, warm in nature, it is related to the liver and stomach channels.

Functions:

Removes blood stasis, arrests bleeding, promotes blood circulation and kills pain.

Applications:

1. To treat various types of internal and external bleeding syndromes:

This herb can not only arrest bleeding, but also dissipate blood stasis, producing outstanding curative effects. It is used for various types of internal and external bleeding syndromes and is especially applicable to bleeding syndromes due to blood stasis. It is an excellent herb with features such as the ability to arrest bleeding without leaving any blood stasis and to dissipate blood stasis without impairing primordial *qi*.

It can take effect when used alone for oral administration and external application. It can also be used in combination with ophicalcite and burnt hair, e.g., Huaxue Dan in the book 'Records of Traditional Chinese and Western Medicine in Combination'.

2. To treat traumatic injuries and blood stasis with pain:

Appendix 5

This herb can promote blood circulation by removing blood stasis to subdue swelling and kill pain, so it is a major traumatological herb

It can be used alone for oral administration or external application or in combination with blood-circulation-promoting and *qi*-activating herbs

3 To treat other syndromes due to blood stasis, such as coronary angina pectoris, ischemic cerebrovascular diseases, cerebral hemorrhagic sequelae, chronic hepatitis due to blood stasis and metroptosis

In recent years, this herb has produced quite good curative effects in the treatment of such ailments as coronary angina pectoris, ischemic cerebrovascular diseases, cerebral hemorrhagic sequelae, etc , as it can dissipate blood stasis It can also be used for chronic hepatitis due to blood stasis

In addition, its injectio has been used for intramuscular injections and it has been made into vaginal suppositories for the treatment of metroptosis

Dosage and Administration:

3-10 g , decoction

1-1 5 g, powder form

In most cases, grind into powder for oral administration It can also be included in a decoction Use an adequate amount externally Grind into powder for external rubbing or mix the powder with oil for external application

Reference Materials:

'The Compendium of Materia Medica'

"Arresting bleeding, dissipating blood stasis and killing pain "

"Treats knife and arrow incised wounds, traumatic bleeding, hematemesis (vomiting blood), epistaxis (nosebleed), hematochezia, dysentery with bloody dysentery, metrorrhagia (profuse uterine bleeding especially between menstrual periods), incessant menstruation, postpartum lochiostasis, swooning due to excessive loss of blood during child delivery, pain due to blood stasis, conjunctivitis, tiger, snake and other animal bites "

'The Records of Traditional Chinese and Western Medicine in Combination'

"This herb is good at dissipating blood stasis and arresting bleeding due to excessive blood-heat, so it is a major drug for hematemesis and epistaxis After recovery, no blood stasis will remain in the channels It will not impair new blood although it dissipates blood stasis, so it can be characterized as a wonderful herb for the regulation of blood circulation "

Modern Researches:

This herb contains pseudoginseng saponin, flavonoid glycosides, quercetin, quercitrin, beta-sitosterol and B-N-Z-diacetyl-L-A,B-diaminopropionic acid, an active hemostatic ingredient

Appendix 5

Notoginseng is a fairly recent newcomer to Chinese herbalism, the first recorded usage dating from the sixteenth century Nevertheless, it has attained an importance as a tonic medicine that supports the function of the adrenal glands, in particular the production of corticosteroids and male sex hormones It can speed up the elimination of sports fatigue and strengthen the physique It also helps to improve blood flow through the coronary arteries, thus finding use as a treatment for arteriosclerosis, high blood pressure and angina (a disease marked by spasmodic attacks of intense suffocative pain)

It can arrest bleeding It also has anti-inflammatory, analgesic, tranquilizing, anti-ageing and antineoplastic effects

Both notoginseng and ginseng are plants under the same family of Araliaceae There is a popular custom of using notoginseng root (Radix Notoginseng) for tonification

The roots are said to be analgesic, antiinflammatory, antiphlogistic, antiseptic, astringent, cardiotonic, discutient, diuretic, haemostatic, hypoglycaemic, styptic, tonic and vulnerary They are used in the treatment of contused wounds, soft tissue injuries and all kinds of bleeding, both internal and external, like haematuria, epistaxis, haematemesis, uterine bleeding etc They are also used in the treatment of coronary heart disease and angina pectoralis The roots can be applied externally as a poultice in order to help speed the healing of wounds and bruises

There is much confusion in literature over this plant and P pseudo-ginseng It is probable that the two can be used interchangeably but this has still to be confirmed The following are the uses attributed to P pseudo-ginseng

The roots and the flowers are antibacterial, antiinflammatory, antiseptic, cardiotonic, diuretic, haemostatic and hypoglycaemic The root is used internally in the treatment of coronary heart disease and angina The roots are also used both internally and externally in the treatment of nosebleeds, haemorrhages from the lungs, digestive tract and uterus, and injuries The roots are harvested in the autumn, preferably from plants 6-7 years old, and can be used fresh or dried The flowers are used to treat vertigo and dizziness

In addition, the root of gynura (Gynura segetum (Lour) Merr) of Compositae is known as Juye Sanqi (chrysanthemum-leaf notoginseng), the root or whole herb of aizoon stonecrop (Sedum aizoon L) of Crassulaceae is known as Jingtian Sanqi (aizoon pseudo-ginseng) These two herbs have similar functions to those of notoginseng root (Radix Notoginseng), with weaker potency, but they can also remove toxic substances and relieve inflammation

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2.5 棕櫚子 saw palmetto

Origin:

Saw Palmetto is native to the United States South Atlantic coast and Florida, as well as Southern Europe and North Africa. This small palm tree grows to a height of six to ten feet, and has a fan-shaped crown of leaves and dark red berries approximately the size of olives.

Functions:

Traditional indications for the use of Saw Palmetto include: cystitis, chronic bronchitis, asthma, diabetes, dysentery, indigestion, and for "underdeveloped breasts." Modern usage of Saw palmetto is overwhelmingly for the treatment of benign prostatic hyperplasia (BPH).

Clinical studies:

The liposterolic extract of the fruit, standardized to contain at least 85% fatty acids and sterols, is currently used in the treatment of BPH.

In a double-blind, placebo-controlled study of 110 BPH patients, 160mg twice per day of a standardized Serenoa extract significantly improved nocturian, dysuria, post-voiding residual urine, flow rate, patient self-rating, and the physican's overall assessment.

Dosage:

The dose of the standardized liposterolic Serenoa extract (85-95% fatty acids and sterols) used in the majority of clinical studies on BPH is 160mg twice per day or 320mg once daily. Duration of six-month is the minimum to assess clinical efficacy.

Toxicology:

There are no known cases of toxicity. Occasionally, patients experience minor gastrointestinal symptoms (nausea, abdominal pain).

Appendix 5

3 Safety test

3.1 Microbiologic, heavy metals and pesticides

The test article BYSH Sachets was tested by SGS Hong Kong Ltd from April 11 to 20, 2005 Twenty-two sachets (5 5g per sachet) of the samples were collected on April 11 2005 The test items included *heavy metals* (Arsenic 砷, Lead 鉛, Mercury 汞 and Cadmium 鎘), *Pesticides Residue* (殘餘殺蟲劑) and *microbiological tests* 微生物 (total aerobic Microbial Count 細菌總數, total combined moulds & yeast 黴菌和酵母菌計數, and Escherichia coli 大腸桿菌)

Test Item		Result		Reference Standard	Standard Source
		Content	Daily Intake		
Heavy metals	Arsenic(砷)	2mg/kg (ppm)	10μg/day	1500μg/day	Chinese Medicine Council of HK
	Lead(鉛)	0 4mg/kg (ppm)	2μg/day	179μg/day	Chinese Medicine Council of HK
	Mercury(汞)	<0 1mg/kg (ppm)	<0 7μg/day	36μg/day	Chinese Medicine Council of HK
	Cadmium(鎘)	0 3mg/kg (ppm)	1μg/dose	3500μg/dose	Chinese Medicine Council of HK
Pesticides Residue		9 pesticides tested are complied with HK Dept proposed limit for Chinese Proprietary Medicine (oral)			
Microbial examination	Total Aerobic Microbial Count (菌落總數)	<10 colony/g		***1000/g*** (Not contain raw material powder) ***30000/g*** (Contain raw material powder)	Chinese Medicine Council of HK
	Total Combined Moulds & Yeast (黴菌及酵母菌)			100/g	Chinese Medicine Council of HK
	Moulds 黴菌	<10/g	10/g		
	Yeast 酵母菌	<10/g	<10/g		

	Escherichia coli (大腸桿菌)	Absent/g	Absent/10g	Absent/g	Chinese Medicine Council of HK

According to the results, all tested items comply with the requirements of Chinese Medicine Council of Hong Kong, "Proprietary Chinese Medicine" Registration Application Booklet (Aug 2004 version) - heavy metals and toxic elements, microbial limit and pesticides residue

3.2 Animal tests

Herb	Safety / toxicity
白花蛇舌草	Mice LD50 (peritoneal injection) 104g raw herb/kg [1]
薏苡仁	Non-toxic to human body without genetoxicity [2]
黃芩	Oral LD50 20g/kg No maternal toxicity, errbryonic and development toxicity, teratogenicity [3] and mutagenicity[5]
三七	Maximal non-effect level of radix notoginseng powder was10g/kg bw d after 30 days oral administration [4]

4 Literatures on animal tests on the drugs or herbs

4 1 白花蛇舌草

- Anticancer activity
- Anti-mutagenicity effect
- Immunomodulating effects
- Anti-inflammatory effect
- Anti-bacterial effect

4 2 薏苡仁

- Anticancer effect
- Hypoglycemia effect
- Analgesic effect

4 3 黃芩

- Anticancer effect
- Anti-bacteria effect
- Anti-inflammatory effect
- Anti-oxidant effect
- Anti-allergic effect

4 4 三七

- Anti-aging
- Stop bleeding

Appendix 5

- Relieve swelling and alleviate pain
- Reduce myocardial oxygen consumption and lower arterial pressure
- Anti-inflammatory effect

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Appendix 6

ACUTE ORAL TOXICITY STUDY IN MICE

Protocol

Prepared by

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Appendix 6

ACUTE ORAL TOXICITY STUDY IN THE MICE

INTRODUCTION

Objective

The objective of this study is to assess the acute toxicity of a test article when administered as a single oral dose followed by an observation period of 14 days and thereby obtain information both about hazard assessment purposes and for ranking articles.

The test is usually conducted in accordance with the OCED Guideline No. 420, "Acute Oral Toxicity – Fixed Dose Method", July 1992, and the EEC Directive published in: "Official Journal of the European Communities" No: L 383A, volume 35,29. 12. 1992, part B. 1 "Acute Toxicity (Oral) – Fixed Dose Method" or "*Procedures for Toxicological Assessment on Food Safety Acute Toxicity Test GB15193.3-94"* issued by Ministry of Health of China.

Reason for the choice of animal species

Single-dose toxicity tests are usually conducted on at least two mammalian species of known strain using equal number of both sexes. Rodents such as the mouse and rat are suitable for the qualitative study of toxic signs and the quantitative determination of the approximate lethal dose. So the mice are selected as the test model for the present study because of its proven suitability in acute toxicity studies.

Initial considerations

It is the principle of the fixed dose method that in the main study only moderately toxic doses will be used and that administration of doses that are expected to be lethal should be avoided. Also doses that are known to cause marked pain and distress, due to corrosive or severely irritant actions, need not be administered. During the test animals obviously in pain or showing signs of severe distress will be killed.

Principle of the test

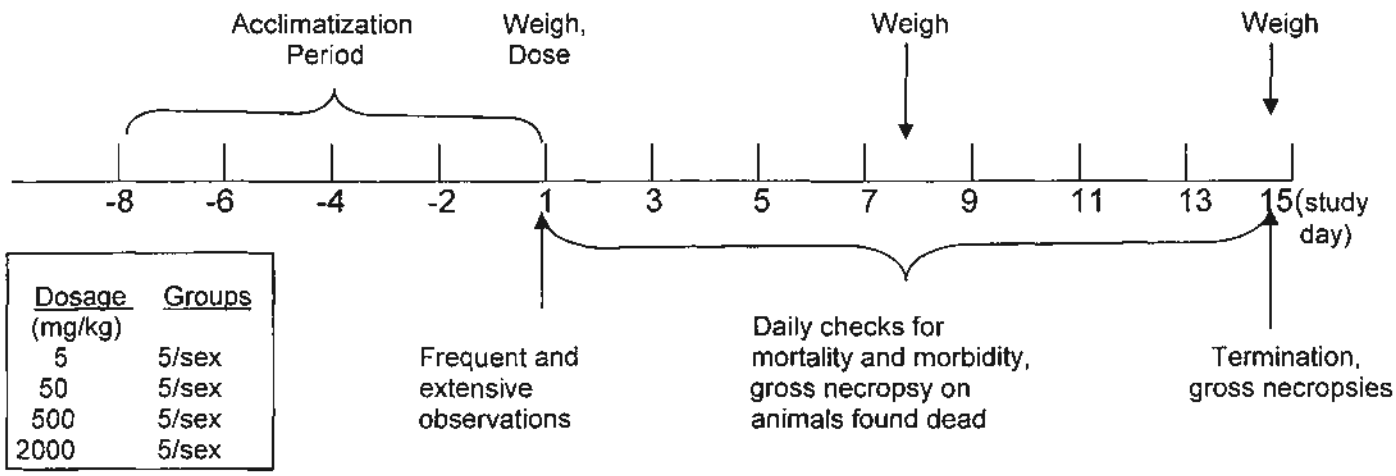
In a preliminary sighting study, the effects of various doses administered to single animals of one sex will be investigated in a sequential manner. The sighting study yields information on the dose-toxicity relationship, including an estimate of the minimum lethal dose. In the main study, the test article is administered to groups of five male and five female animals at one of the fixed doses (5, 50, 500 and 2000 mg/kg). The dose is derived from the sighting study. It is immediately below that which is expected to result in mortality. If evident toxicity is not seen at the chosen dose level, the article will be re-tested at the next higher dose level. If, at the initial dose level, animals die, or a severe toxic reaction requires the removal of animals from the study for animal welfare reasons, the article is re-tested at the next lower dose level.

According to the US EPA pesticides that do not produce evident toxicity at a lower dose level

Appendix 6
should be tested at a dose level of 5000mg/kg.

MATERIALS AND METHODS

Line Chart for the design and conduct of an acute systemic toxicity study:



Test article

Prior to the commencement of the study, the Sponsor will supply the test article, and a Test Article Characterisation Sheet indicating test material identity, stability, appearance, handling and safety instructions and storage conditions, etc.

The test article will be dissolved or suspended in a non-toxic vehicle i.e. sterile water, a vegetable oil (i.e. sesame oil) or carboxymethylcellulose solution (CMC).

Animals

The experiment will be performed in mice (usually NIH or KM strain). The mice will be 6 to 8 weeks old and the weight will be about 20 g. The range of weight variation will not exceed ± 10% of the mean weight. Equal number of both sexes will be used.

An acclimatisation period of at least 5 days will be allowed.

Housing

The study will take place in an animal room with filtered air at a temperature of $21^0C \pm 3^0C$ and relative humidity of 55% ± 15%. The room has been designed to give 10 air changes per hour. The room will be illuminated to give a cycle of 12 hours light and 12 hours darkness. Light will be on from 0600h to 1800 h.

The temperature and relative humidity in the animal room will be recorded hourly during the study and the record will be retained.

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The rats will be kept in transparent polycarbonate cages (floor area: 810 cm^2) with two or three in each cage, males and females separated. The cages will be cleaned and the bedding changed at least twice a week.

Before the animals arrive, the animal room will be cleaned. During the study the animal room will be cleaned regularly.

Bedding

The bedding will be softwood sawdust. Regular analyses for relevant possible contaminants are performed. Certificates of analysis are retained.

Diet

A complete pelleted rodent diet (for growing animals) from qualified supplier, will be available *ad libitum*. Analyses for major nutritive components and relevant possible contaminants are required.

On the day of dosing the mice will have fasted over night and the food will be withheld for a further 3 hours period after dosing.

Drinking water

The animals will have free access to bottles with domestic quality drinking water acidified with hydrochloric acid to pH 2.5 in order to prevent microbial growth. Analyses for possible relevant contaminants are required to perform regularly.

Animal identification and grouping

On the day of arrival each animal will be identified by ink on different part of the mouse body. The animals will be weighed and allocated to the groups.

Each cage will be identified by a colour coded cage card marked with study number, group number, sex and animal ear numbers.

In the sighting study one animal will be used at each necessary dose level. Usually, females will be used unless other information suggests that males will be most sensitive sex.

In the main study each group used will consist of five males and five females mice.

Dose administration

A single oral dose will be administered by gavage. The volume will be less than 0.2 ml/10g body weight. If the vehicle is water 0.4 ml/10g body weight is accepted.

TESTING PROCEDURE

Sighting study

Appendix 6
The effects of various doses will be investigated in single animals. Usually, female animals will be used in the absence of information derived from structure-activity relationships or other information indicating that males will be the more sensitive sex. Dosing will be sequential, allowing at least 24 hours before dosing the next animal. All animals will be carefully observed for signs of toxicity for at least 14 days. The following initial dose levels will be considered: 5, 50, 500 and 2000 mg/kg.

The initial dose level will be selected according to information from related chemicals and information from the Sponsor. In the absence of such information, 500 mg/kg will be used as the initial dose. If no signs of toxicity are seen after the initial dose, the next higher dose level will be investigated. If no mortality occurs at 2000 mg/kg, the sighting test will be complete and the main study will be conducted at this dose level. If severe effects, necessitating humane killing are seen at the initial dose (e.g. 500 mg/kg), the next lower dose (e.g. 50 mg/kg) will be given to another animal. If this animal survives, further animals may be dosed with the appropriate intermediate dose levels between the fixed doses. Usually, not more than 5 animals will be used in this procedure.

Main study

Number of animals and dose levels

10 animals (five males and five females) will be used for each dose level investigated. The dose level to be used in the main study will be selected from one of four levels, 5, 50, 500 and 2000 mg/kg. On the basis of the results from the sighting study the dose likely to produce evident toxicity but no mortality will be selected. If the data from the sighting study suggest that mortality will occur at 5 mg/kg, the test article can be investigated at a lower dose level.

In most cases it is likely that the data obtained from the sighting study will be adequate to allow the appropriate dose level to be selected for the main study (i.e. a level that produces evident toxicity but no mortality). However, if evident toxicity is not seen at the selected dose level, the article will be re-tested at the next higher level, unless mortality has already occurred in the next higher level. If animal die at the initial dose chosen, or a severe reaction requires removal of animals from the study for animal welfare reasons, the article will be re-tested at the next lower dose level.

Clinical signs

Each mouse will be observed 1, 3, and 6 hours after administration and then once daily over a period of at least 14 days. All signs of ill health and behavioral changes will be recorded.

Body weight

Body weight will be recorded prior to dosing (day 1) and days 2, 3, 4, 8 and 15. Any mouse found dead from day 2 to 15 will be weighed before necropsy. The group mean weight will be calculated.

Mortality and necropsy

Any rat found dead during the 14-day observation period will be subjected to gross necropsy examination. The time of death will be recorded as precisely as possible. Animals showing

severe and ending signs of distress and pain during the observation period will be killed by inhalation of a high concentration of CO_2 and necropsied. All mice surviving on day 15 will be killed by inhalation of a high concentration of CO_2 or by an intraperitoneal injection of 5% Mebumal® and subjected to a gross necropsy examination.

RESULTS

Evaluation

The dose level that produces evident toxicity will be identified. The interpretation of results including the estimated minimum lethal dose, and the highest dose level that do not produce mortality will be discussed.

The interpretation of the results will be made according to Annex 2 in the OECD Guideline and Section 3.2. in the EEC Guideline. The contents are included in this standard protocol (Appendix I) as well as in the report.

Reports

Two copies of the final report will be issued.

Archives

For a period of 5 years the following material relating to the study will be retained in the archives of the Clinical Trials Section Institute of Chinese Medicine The Chinese University of Hong Kong

Protocol, protocol supplements and correspondence
Test material receipts
Animal records
All original data
Final report

At the end of the storage period ICM will contact the Sponsor for instructions whether the material should be transferred, retained or destroyed. Implementation of such instructions will be at additional costs to the Sponsor.

Appendix 6

Appendix I

INTRPRETATION OF RESULTS

DOSE	RESULT	INTERPRETATION
5 mg/kg*	Less than 100% survival**	Compounds which may be very toxic (ie with LD_{50} values of approx. 25 mg/kg or less) if swallowed.
	100% survival, but evident toxicity	Compounds which may be toxic (ie with LD_{50} values between approx. 25 mg/kg and approx. 200 mg/kg) if swallowed.
	100% survival, no evident toxicity	Test at 50 mg/kg if not already tested at that dose.
50 mg/kg	Less than 100% survival **	Compounds which may be toxic or very toxic if swallowed. Test at 5 mg/kg if not already tested at that dose level.
	100% survival, but evident toxicity	Compounds which may be harmful (ie with LD_{50} values between approx. 200 mg/kg and 2000 mg/kg) if swallowed.
	100% survival, no evident toxicity	Test at 500 mg/kg if not already tested at that dose.
500 mg/kg	Less than 100% survival**	Compounds which may be toxic or harmful if swallowed. Test at 50 mg/kg if not already tested at that dose level.
	100% survival, but evident toxicity	Compounds with LD_{50} values above approx. 2000 mg/kg but which may be of some concern due to the nature of the toxic effects.
	100% survival, no evident toxicity	Test at 2000 mg/kg if not already tested at that dose.
2000 mg/kg	Less than 100% survival**	Compounds which may be of some concern if swallowed. Test at 500 mg/kg if not already tested at that dose level.
	100% survival, with or without evident toxicity	Compounds which do not present a significant acute toxic risk if swallowed.

* Where a dose of 5 mg/kg produces significant mortality, or where a sighting study suggests that mortality will result at that dose level, the test article should be investigated at a lower dose level. The level chosen should be that which is likely to produce evident toxicity but no mortality.

** Includes compound related mortality and humane kills but not accidental deaths.

NOTE: Interpretation has been given with respect to the data obtained in the extensive validation studies of the method and relates to approximate LD_{50} values in the range below 25 mg/kg, 25-200 mg/kg, 200-2000 mg/kg and above 2000 mg/kg. However the results can be used for other ranges by consideration of both the data from the sighting study and the main study, with judgement as to the whether the interpretation given here is adequate (bearing in mind the natural variability in the LD_{50} value and of the slope of the dose-response curve) or whether any adjustment is necessary.

Appendix 6

Appendix II

Acute observation record

			(mg/kg)		
Species	Sex	Route	Dose level	Animal code	Date dosed

Study date	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No signs observed														
Reduced motor activity														
Ataxia														
Lost righting reflex														
Convulsions														
Death observed														

SGS Hong Kong Ltd. Appendix 7
Retail & Supply Support Division

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CUCAMED COMPANY LIMITED
THE CHINESE UNIVERSITY OF HONG KONG
SHATIN N.T. HONG KONG

Job No. :1316327

Acute Oral Toxicity Study on the submitted sample identified by the client as Danggui capsules

Summary

The acute oral toxicity of **Danggui capsules** in mice was determined according to the method recommended in the "*Procedures and methods for toxicological assessment on food safety---Acute toxicity test (GB15193.3-94)*" issued by Ministry of Health (MOH), Peoples Republic of China.

When tested as specified herein, the submitted test article identified by the client as **Danggui capsules** did not induce any mortality in laboratory animals following oral administration of 20000mg/kg and was considered to have an acute oral LD50 value greater than 20000mg/kg.

Under the experimental conditions described in the report, the submitted sample identified by the client as **Danggui capsules** was considered to be non-toxic to laboratory animals in accordance with *Procedures and Methods for Toxicological Assessment on Food Safety---Acute Toxicity Test (GB 15193.3-94)*, People's Republic of China.

While the term non-toxic is not defined by any scientific body or regulatory agency, in general, Toxicological Assessment on Food Safety issued by Ministry of Health (MOH) Peoples Republic of China (PRC) or World Health Organization (WHO) recognize substances as being acutely " toxic" if the test article induces mortality in animals administered doses at up to 5.0g/kg.

****

Signed for and on behalf of
SGS Hong Kong Ltd.

TAMMY CHENG
DEPUTY TECHNICAL DIRECTOR - HEALTHCARE AND PHARMACEUTICAL SERVICES

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Introduction and Purpose

The purpose of this safety test is to determine if acute health hazards are associated with ingestion of the test article. The measure of acute toxicity can be expressed as the median lethal dose (LD50), a statistically derived value that estimates the dose that would theoretically kill 50% of the test animal group. Such tests require the dosing of a relatively large number of animals to generate precise LD50 values.

Often such a precise measurement of lethality is either not required to characterise the test article or may not be practical as the test article may be minimally toxic to animals following oral administration. To minimise the number of animals used in acute oral toxicity tests without compromising the intent of such safety tests, the use of limited screening tests with the administration of a single, high limit dose to a group of animals is often adequate for assessing the inherent acute toxicity of the test article.

The test was conducted in accordance with the procedures as outlined in :

Procedures for Toxicological Assessment on Food Safety
Acute Toxicity Test
GB15193.3-94

The study was planned and monitored by SGS Hong Kong Ltd Healthcare & Pharmaceutical Services, 5/F-8/F., Metropole Square, 2 On Yiu Street, Siu Lek Yuen, Shatin, Hong Kong and performed by Ministry of Health (MOH) approved site during March 12 – 19, 2003.

Testing Regime

The Client requested characterisation of the acute oral toxicity of the submitted samples. These data were established through the use of acute oral toxicity upper limit test.

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Test Article

Product Description	:	Danggui capsules
Quantity Received	:	5 bottles (450's)
SGS Sample No.	:	1316327-101
Sample Receiving Condition	:	In unopened plastic bottle under ambient condition
Sample Receiving Date	:	14 February 2003

Test article characterisation (purity, solubility and stability, etc.) was the responsibility of the client. The test article was labeled with laboratory number of this study and kept in room temperature.

Test Animals

Strain	:	NIH mice (male, female)
Source	:	Guangdong Provincial Medical Laboratory Animal Supply Centre
Date(s) Received	:	7 March 2003

Upon arrival, an equal number of male and female (n = 20) were randomly assigned to a control group or treatment group. Animals were housed by sex in the observation battery rack and stained the hair on different part of the body for animal identification. Animals were observed for at least 5 days for signs of illness or disease prior to initiating tests.

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Procedure

Acute Oral Toxicity Test
White NIH mice (male and female, Quality Certificate No.: 2001A044) each weighing between 19 and 22 grams were selected for each dosage. The animals were housed in plastic cages with stainless steel wire mesh caps, the floors of the cages were put with softwood sawdust. The temperature of animal room was 20 ± 2°C, humidity 50 – 60%. The room was illuminated to give a cycle of 12 hours light and 12 hours darkness. Animals were maintained on a commercial mouse food diet and water was available *ad libitum*. Twelve hours prior to dosing, all food was removed to fast the animals before initiating the test. On the day of the test, animals were identified and body weights recorded. The dosage to be administered was calculated based on the animal's body weight. The total dosage was up to 20g/kg body weight. The maximum volume is 0.4ml/10g body weight. The control group was treated with same amount of distilled water.

For test articles that are liquids or could be administered as solutions, suspensions or extracts, appropriate doses were administered to animals using a feeding needle and syringe. For certain solid-form test articles, doses were administered by incorporating the material into a feed mix that was fed to laboratory animals over 24 hours period. The method of sample administration used for the submitted test article is outlined in the Sample Preparation section of this report.

Animals were closely observed for gross toxicological effects immediately after a single dose administration of the sample and then daily for a 7-day observation period. Test animals' body weights, a sensitive indicator of toxic insult, were recorded during the observation period. Necropsies of dead, moribund or surviving animals were performed if indicated during the progression of the study.

Sample Preparation

A test solution was prepared by dissolving the test sample with distilled water to a concentration of 500mg/ml. A single dose administration of 0.4ml/10g body weight using needle and syringe was given. The control group was treated with same amount of distilled water.

Clinical signs

Each mouse was observed at 1, 3 and 6 hours after administration during Day 1 and thereafter daily for a period of 7 consecutive days. The observation items included body weight, behavior and mortality.

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Statistical Analysis

Data were analyzed using student t-test for the data of body weight with SPSS 10.0 for Window.

Results

40 NIH mice (20 male, 20 female) were administered an oral dose of the test article 20,000mg/kg.

Testing Period : 12 – 19 March 2003

Table 1 Mortality and Body Weight Changes after oral Administration of **Danggui capsules**

Group	No. of Animals	Dose (g/kg)	Mortality %	Average Body Weight (g)		Body Weight Gain	P Value
				Initial	Final		
Test Group	M 10	20	0	19.45±0.54	29.59±2.32	10.14±2.35	>0.05
	F 10	20	0	20.10±0.98	29.19±2.12	9.09±1.69	>0.05
Control Group	M 10	--	0	20.20±0.98	30.21±1.92	10.01±1.91	
	F 10	--	0	19.84±1.23	29.12±2.02	9.28±1.85	

Observations

All animals appeared normal throughout the 7-days observation period. There were no differences of body weights between test group and control group (P > 0.05).

Conclusion

When tested as specified, the submitted test article identified by the client to be used for **Danggui capsules** was considered to be essentially **non-toxic** to laboratory animals following oral administration at 20,000mg/kg (Table 2).

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Table 2 Classification of Chemical Toxicity (Oral) From GB 15193.3--94

Category	LD50 (mg/Kg)
Extremely toxic	1.0 or less
Highly toxic	1.0 – 50.0
Moderately toxic	51.0 – 500.0
Slightly toxic	501.0 – 5000.0
Practically non-toxic	5001.0 – 15,000.0
Non-toxic	>15,000

*** End of Report ***

A 90 DAY ORAL TOXICITY STUDY IN RATS

Protocol

Objective

The objective of this study is to assess the chronic and systemic toxicity of the herbal formula on the rat when administered for a period of 90 days.

The present study will be conducted in accordance with the CPMP Guidelines (1983), which are the guidelines accepted by the EU (Guidelines on the quality, safety and efficacy of medicinal products, January 1989).

Reason for the choice of animal species, route of administration and dose levels

The rat will be used as the test model because of its proven suitability in toxicology studies.

Oral treatment is chosen in order to comply with the intended clinical route of administration.

Study timetable (to be amended)

Arrival of animals
Commencement of dosing
Live animal work complete
Draft report to the Sponsor

Animals

The experiment will be performed in 80 SPF Sprague Dawley rats (40 males and 40 females). At start of the acclimatisation period, the rats will be 4 to 5 weeks old and the weight will be 70-90 g. Ten animals (5 of each sex) will be available until completion of the acclimatisation period for replacement purposes.

An acclimatisation period of at least 5 days will be allowed in order to reject animals in poor condition or at extremes of the weight range.

Housing

The study will take place in an animal room provided with filtered air at a temperature of 22^0 C ± 3^0C, with relative humidity of 55% ± 15%. The room will be illuminated to give a cycle of 12 hours light and 12 hours darkness. Light will be on from 0600 to 1800h.

The temperature and relative humidity in the animal room will be recorded hourly during the study and the records will be retained.

Bedding

The bedding will be softwood sawdust. Regular changes of the sawdust are required.

Diet

A complete pelleted rodent diet will be available *ad libitum* throughout the study. Analyses for major nutritive components and relevant possible contaminants are performed regularly on the diet.

Drinking water

The animals will have free access to bottles with domestic quality drinking water that acidified with hydrochloric acid to pH 2.5 in order to prevent microbial growth. Analyses for possible relevant contaminants are performed regularly.

Animal randomisation and allocation

On the day of arrival the animals will be allocated randomly to for groups and a group of extra animals using a randomisation scheme.

Prior to commencement of dosing the animals may be re-allocated in order to reduce possible intergroup mean body weight differences. Data available from predosing observations will be included in the re-allocation.

Animal and cage identification

Each animal will be identified by punched earmarks.

Each cage will be identified by a colour coded cage card marked with study number (Lab No), cage number, group number, sex and animal number.

Treatment

The groups, dose levels, animal numbers and colour codes will be as follows:

Group	Dose Levels (g/kg/day)	Animal Numbers		Colour code
		Males	Females	
1	0 (vehicle)	1-10	11-20	White
2	1.2	21-30	31-40	Blue
3	2.8	41-50	51-60	Green
4	6	61-70	71-80	Red

The dose will be given orally by gavage bid according to the most recent body weight. The animals will be treated daily for at least 90 days and until the day before necropsy.

The dose volume will be 2 ml/100g body weight.

The first day of dosing will be designated as day 1.

Control of dose formulation calculations and preparations

Before preparation of dose formulation the calculations on which the preparations will be based, will be checked independently by two persons.

Each step of the dose formulation procedure will be documented by weighing.

Each dose formulation will be weighed before and after dosing, and the amount of dose formulation used for each group will be compared with predicted daily usage.

Clinical signs

All visible signs of ill health and any behavioural changes will be recorded daily. Any deviation from normal will be recorded with respect to time of onset, duration and

intensity.

Mortality

If an animal dies or is killed for ethical reasons during the study the animal will be necropsied and subjected to the procedures described in "Terminal observations".

Any decision regarding sacrifice for ethical reasons will be taken by the Study Director.

Body weight

All animals will be weighed on arrival, on the first day of dosing (day 1) and weekly thereafter. Also, the weight at necropsy will be recorded.

Food consumption

From start of dosing, consumption of food will be recorded weekly for each cage.

Laboratory investigations

Blood samples

During the last week of dosing, blood samples will be taken from all animals. Blood samples will be drawn from the orbital venous plexus during CO_2 anaesthesia. For haematology 750 µl EDTA stabilised blood will be taken, for the coagulation tests, 750 µl citrate stabilised blood. Twenty µl blood will be taken with micropipette with dry Na-heparin for the blood glucose analysis. Blood samples for clinical chemistry (3 ml samples) will be taken into plain glasses for serum. The samples will be analysed on the day of collection. Remains of the serum samples will be frozen and stored at approx. -18^0C for possible re-analyses.

Haematology

Parameter	Abbreviation	Unit
Total white blood cell count	WBC	10^9/l
Total red blood cell count	RBC	10^{12}/l
Lymphocyte	Lympho	10^9/l

Monocyte	Mono	10^9/l
Neutrophil	Neutro	10^9/l
Hemoglobin	Hb	g/l
Platelet	PLT	10^3/ul
Thrombin time	TT	Sec
Parameter	Abbreviation	Unit
Total white blood cell count	WBC	10^9/l
Total red blood cell count	RBC	10^{12}/l
Lymphocyte	Lympho	10^9/l
Monocyte	Mono	10^9/l

Clinical chemistry

Parameter	Abbreviation	Unit
Alanime aminotransferase	ALT	u/l
Aspartate aminotransferase	AST	u/l
Alkaline phosphatase	ALP	u/l
Total protein	TP	g/dl
Albumin	ALB	g/dl
Total bilirubin	T-Bil	mg/dl
Total Cholesterol	T-CHOL	mg/dl
High density Lipoprotein-Cholesterol	HDL-CHOL	mg/dl
Low density Lipoprotein-Cholesterol	LDL-CHOL	mg/dl
Blood urea nitrogen	BUN	mg/dl
Creatinine	Crea	mg/dl
Clucose	Glu	mg/dl
Parameter	Abbreviation	Unit
Alanime aminotransferase	ALT	u/l
Aspartate aminotransferase	AST	u/l
Alkaline phosphatase	ALP	u/l
Total protein	TP	g/dl
Albumin	ALB	g/dl
Total bilirubin	T-Bil	mg/dl

Terminal observations

On the day of necropsy the animals will be weighed, examined externally,

anaesthetised with an intraperitoneal injection of a barbiturate and sacrificed by exsanguination. The animals will be sacrificed and necropsied in the sequence of one or two animals/group.

Necropsy

A macroscopic examination will be performed after opening the cranial, thoracic and abdominal cavities and by observing the appearance of the organs and tissues *in situ*. Any macroscopic change will be recorded with details of the location, colour, shape and size.

Organs and tissues

Either whole organs or selected samples of the indicated organs and tissues will be subjected to the procedures itemised in the list given below. Weights will be recorded in the PathData computer system.

Organs and tissues	Weigh	Fix	Micro	Organs and tissues	Weigh	Fix	Micro
Abnormalities (gross lesions)		x		Ovaries	x	x	
Adrenals	x	x		Pancreas		x	
Aorta (thoracic)		x		Pituitary	x	x	
Brain (cerebrum, cerebellum, brain stem)	x	x		Prostate	x	x	
Epididymides	x	x		Salivary gland (right submandibular)		x	
Eyes and with lens/optic nerve		x		Sciatic nerve		x	
Femur (right)		x		Seminal vesicles	x	x	
Harderian glands		x		Skin		x	
Heart	x	x		Spinal cord (cervical, midthoracic and lumbar)		x	
Intestine small (duodenum, jejunum, ileum)		x		Spleen	x	x	
Intestine large (caecum, colon, rectum)		x		Sternum for bone marrow		x	
Kidneys	x	x	x	Stomach (glandular, non glandular)		x	x
Liver	x	x	x	Testes	x	x	
Lungs	x	x		Thymus	x	x	

Mammary gland (caudal)		x		Thyroids (incl. Parathyroid)	x	x	
Mandibular lymph nodes	x	x		Trachea		x	
Mesenteric lymph node		x		Urinary bladder		x	
Muscle (quadriceps femoris, right)		x		Uterus (horn, cervix)	x	x	
Oesophagus		x		Vagina		x	

Pared organs will be weighed together. The relative organ weights, i.e. the organ weight as a percentage of the body weight, will be calculated for each animal.

All tissues will be initially fixed in phosphate buffered neutral 4% formaldehyde with the exception of the eyes (Davidson's fixative) and testes (Bouins fixative). The fixative for long term preservation will be phosphate buffered neutal 4% formaldehyde for all tissues. The lungs will be infused with fixative at necropsy.

Processing and microscopic examination

After fixation, the organs and tissues sampled for microscopic examination will be trimmed and representative specimens will be taken for histological processing. The specimens will be embedded in paraffin and cut at a normal thickness of 4-5 µm, stained with haematoxylin and eosin and examined under a light microscope.

Histological alterations will be graded on a 5 grade system:

Grade 1 - Minimal/Very few /Very small
Grade 2 - Slight/Few/Small
Grade 3 - Moderate/Moderate number/Moderate size
Grade 4 - Marked/Many/Large
Grade 5 - Massive/Extensive number/Extensive size
Present - Finding present/ Severity not scored

The following organs and tissues will be examined microscopically:

1) Liver, kidney and bone from all control (group 1)
   And high dose animals (group4)
2) All organs and tissues from all animals dying or killed during the study.
3) All gross lesions from all animals.

Peer review

A peer review will be performed on slides from liver, kidneys and bone of two males and two females from the high dose (group 4) and control group (group 1). Diagnostic discrepancies will be resolved by discussion.

Statistics

Data will be processed to give group mean values and standard deviations where appropriate. Thereafter each continues variable will be tested for homogeneity of variance with Bartlett's test. If the variance is homogeneous, analysis of variance will be carried out for the variable. If any significant difference are detected, possible intergroup differences will be assessed with Dunnett's test. If the variance is heterogeneous, each variable will be tested for normality by the Shapiro-Wilk method. In case of normal distribution, possible itergroup differences will be identified with Student's t-test. Otherwise the possible intergroup differences will be assessed by Kruskal-Wallis's test. If any significant intergroup differences are detected, the subsequent identification of the groups will be carried out with Wilcoxon Rank-Sum test.

Appendix 9

INSTITUTE OF CHINESE MEDICINE
THE CHINESE UNIVERSITY OF HONG KONG
SHATIN,
NEW TERRITORIES
HONG KONG

Job No 1339398

90-day Oral Toxicity Study in on the submitted sample identified by the client as Danggui Capsule

Summary

The objective of this study was to assess the chronic and systemic toxicity of Danggui Capsule in the rat when administered orally for a period of 90 days The study was performed in accordance with the *Guidelines of Pre-clinical Study for New Traditional Chinese Medicine* (《中药临床前研究指导原则》) issued by the Ministry of Health, PR China

Sixty-five Sprague-Dawley (SD) rats (32 males and 33 females) were allocated to three groups The animals were treated daily by oral (gavage) administration for 90 days with concentrations of 0, 0 6 or 3 0 g Danggui Capsule/kg body weight, which are equivalent to 10 and 50 times of human application dose separately The vehicle for suspension of the test article was distilled water The dose volume was 1 ml per 100 g b wt

Clinical signs were recorded daily Body weight was recorded weekly Parameters of haematology and clinical chemistry were assessed for all animals shortly before termination of treatment

On the day of necropsy, the animals were examined externally and sacrificed by exsanguination A macroscopic examination of the cranial, thoracic and abdominal cavities was performed, selected organs/tissues were weighed and selected organs were fixed and processed for microscopic examination

Necropsy at termination revealed no observable gross lesions No abnormal clinical signs were observed No changes in body weight could be related to treatment No treatment related findings were present in parameters of haematology, clinical chemistry No treatment related effects on organ weights were found nor could any microscopic findings be related to treatment

In conclusion, no treatment related effects were seen in rats after ninety days of oral treatment with Danggui Capsule at concentrations of 0 6 or 3 0 g/kg body weight/day

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Objective

The objective of this study was to assess the chronic and systemic toxicity of Danggui Capsule in the rat when administered for a period of 90 days

The present protocol was in accordance with the *Guidelines of Pre-clinical Study for New Traditional Chinese Medicine* (《中药临床前研究指导原则》) issued by the Ministry of Health, PR China

The rat was used as the test model because of its proven suitability in toxicology studies Oral treatment was chosen in order to comply with the intended clinical route of administration The dose levels were selected based on the clinical application

The animals arrived on 30 July 2003 Treatment started on 6 August 2003 and the live animal work was completed on 5 November 2003

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MATERIALS AND METHODS

Test article and vehicle

The test article, Danggui Capsule was supplied by Institute of Chinese Medicines, CUHK in capsules The test article was stored at room temperature The test article was dissolved in distilled water before oral administration

Animals

The experiment was performed in 65 SD rats Clean Animal Grade (32 males and 33 females) At the start of the acclimatisation period, the rats were 5 to 6 weeks old and in the weight range 180-210 g

An acclimatisation period of 7 days was allowed in order to reject animals in poor condition or at extremes of the weight range

Housing

The study took place in the room at a temperature of 25°C ± 2°C, relative humidity of 55% ± 15% The room was illuminated to give a cycle of 12 hours light and 12 hours darkness Light was on from 08 00 to 20 00 h
The rats were kept in transparent polycarbonate cages with twenty to thirty animals in each cage, males and females were separated The cages were cleaned and the bedding changed at least twice per week Before the animals arrived, the animal room was cleaned

Bedding

The bedding was softwood sawdust provided by animal supply center and changed at least twice per week

Diet

Rodent diet provided by Beijing Auxieli Diet Company was available *ad libitum* throughout the study The general consumption of the forage per rat was about 15 - 20g/day

Drinking water

The animals had free access to bottles with filtered sterile drinking water The water was changed daily and the bottles were cleaned weekly

Animal randomisation and allocation

On the day of arrival the animals were allocated to three groups using a randomized procedure based on body weight

Animal and cage identification

Animals were individually identified with their allocation number by dyeing 3% picric acid on the rat's fur

Each cage was identified by a colour coded cage card marked with study number, cage number, group number, sex and animal number

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Treatment
The groups, dose levels and animal numbers were as follows:

Group	Dose levels (g/kg b.wt./day)	Dose volume (ml./100g)	Animal Numbers	
			Males	Females
1	0 (vehicle)	10	1-6	7-13
2	0.6	10	66-79	80-93
3	3.0	10	94-107	108-121

The treatment was given orally by gavage according to the most recent body weight. The animals were treated daily for 90 days and until the day before necropsy.

The dose volume was 1 ml/100 g body weight.

The first day of treatment was designated day 1.

Preparation of dose formulations
Dose formulations were prepared by dissolving the test article in distilled water. New dose formulations were prepared every day.

Clinical signs
All visible signs of ill health and any behavioural changes were recorded daily. Any deviation from normal was recorded with respect to time of onset, duration and intensity.

Body weight
All animals were weighed on arrival, on the first day of treatment (day 1) and weekly thereafter. Also, the weight at necropsy was recorded.

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Laboratory investigations

Blood samples

During the last week of treatment, blood samples were taken from all animals Blood samples were drawn from the tail artery For haematology approximately 300 µl EDTA stabilised blood was taken Twenty µl blood was taken with micropipette with dry Na-heparin for the blood glucose analysis As much blood as possible was taken for clinical chemistry in plain glass tubes for serum The samples were analysed on the day of collection Remains of the serum samples were frozen and stored at approx -18°C

The parameters, methods and units for the laboratory investigations are stated below

Haematology

Parameter	Method/Equipment	Unit
White blood cell count (WBC)	Auto-Hematocyte counter MEK 6318K	10^9/L
Red blood cell count (RBC)	Auto-Hematocyte counter MEK 6318K	10^{12}/L
Haemoglobin (Hb)	Auto-Hematocyte counter MEK 6318K	g/L
Hematocrit (HCT)	Auto-Hematocyte counter MEK 6318K	%
Mean corpuscular volume (MCV)	Auto-Hematocyte counter MEK 6318K	%
Mean cell hemoglobin (MCH)	Auto-Hematocyte counter MEK 6318K	fL
Mean corpuscular hemoglobin concentration (MCHC)	Auto-Hematocyte counter MEK 6318K	pg
LY%	Auto-Hematocyte counter MEK 6318K	g/dL
MO%	Auto-Hematocyte counter MEK 6318K	%
GR%	Auto-Hematocyte counter MEK 6318K	%
Platelet count (Plt)	Auto-Hematocyte counter MEK 6318K	%
Lymphocyte (LY)	Auto-Hematocyte counter MEK 6318K	10^9/L
Mononuclear leucocyte (MO)	Auto-Hematocyte counter MEK 6318K	10^9/L
Granulocyte (GR)	Auto-Hematocyte counter MEK 6318K	10^9/L
RDW	Auto-Hematocyte counter MEK 6318K	10^9/L
PCT	Auto-Hematocyte counter MEK 6318K	%
Mean Platelet volume (MPV)	Auto-Hematocyte counter MEK 6318K	%
PDW	Auto-Hematocyte counter MEK 6318K	fL

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Clinical chemistry

Parameter	Method/Equipment	Unit
Alanine aminotransferase (ALT)	Automatic Analyzer RA 1000 III	U/L
Aspartate aminotransferase (AST)	Automatic Analyzer RA 1000 III	U/L
Total Protein (TP)	Automatic Analyzer RA 1000 III	g/L
Albumin (ALB)	Automatic Analyzer RA 1000 III	g/L
Total Cholesterol (CHOL)	Automatic Analyzer RA 1000 III	mmol/L
Blood Urea Nitrogen (BUN)	Automatic Analyzer RA 1000 III	mmol/L
Creatinine (Crea)	Automatic Analyzer RA 1000 III	µmol /L
Glucose (GLU)	Automatic Analyzer RA 1000 III	mmol/L
Triglycerides (TG)	Automatic Analyzer RA 1000 III	mmol/L

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Terminal observations

On the day of necropsy the animals were weighed, examined externally and sacrificed by exsanguination The animals were sacrificed and necropsied in the sequence of one or two animals/group.

Necropsy

A macroscopic examination was performed after opening the cranial, thoracic and abdominal cavities and by observing the appearance of the organs and tissues *in situ*. Any macroscopic change was recorded with details of the location, colour, shape and size.

Organs and tissues

Either whole organs or selected samples of the indicated organs and tissues were subjected to the procedures itemized in the list given below. Weights were recorded in computer.

Organs and tissues	Organ / Body weight Ratio	Micro
Abnormalities (gross lesions)	X	X
Heart	X	X
Liver	X	X
Spleen	X	X
Lungs	X	X
Kidneys	X	X
Ovaries		X
Testes		X

Paired organs were weighed together. The relative organ weights, i.e. the organ weight as a percentage of the body weight, was calculated for each animal.

All tissues were initially fixed in phosphate buffered neutral 5% formaldehyde with the exception of testes (Bouins fixative). The fixative for long-term preservation was phosphate buffered neutral 5% formaldehyde for all tissues. The lungs were infused with fixative at necropsy.

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Processing and microscopic examination

After fixation, the organs and tissues sampled for microscopic examination were trimmed and representative specimens were taken for histological processing The specimens were embedded in paraffin and cut at a nominal thickness of approximately 5 µm, stained with haematoxylin and eosin and examined under a light microscope

Histological alterations were graded on a 5-grade system

Grade 1 - Minimal/Very few/Very small
Grade 2 - Slight/Few/Small
Grade 3 - Moderate/Moderate number/Moderate size
Grade 4 - Marked/Many/Large
Grade 5 - Massive/Extensive number/Extensive size
Present - Finding present/Severity not scored

Statistics

Data were processed to give group mean values and standard deviations where appropriate Thereafter each continuous variable was tested for homogeneity of variance with Bartlett's test If the variance was homogeneous, analysis of variance were carried out for the variable If any significant differences were detected, possible intergroup differences were assessed with Dunnett's test If the variance was heterogeneous, each variable was tested for normality by the Shapiro-Wilk method In case of normal distribution, possible intergroup differences were identified with Student's t-test Otherwise the possible intergroup differences were assessed by Kruskal-Wallis's test If any significant intergroup differences were detected, the subsequent identification of the groups was carried out with Wilcoxon Rank-Sum test Ranked type of urinalysis data were analysed with Wilcoxon Rank-Sum test

Group differences with an error probability of less than 5% ($p<0.05$) were considered statistically significant

The statistical analyses were made with SPSS 10 0 for Windows

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RESULTS

Clinical signs
All animals survived to the end of treatment or recovery, and no general clinical signs treatment related were noted in the treated groups when compared with controls during treatment or the recovery periods

Body weight (Figure 1, 2, 3, 4 Table 1, 2, 3, 4)
Body weights were unremarkable No significant differences in the mean body weights were observed between treatment groups of either males or females
During recovery period, there were no differences of body weight between treatment groups and control group

Haematology (Table 9, 10, 11,12)
In males, platelets was significantly increased in the 3 0g/kg group, and MCHC value was also increased in comparison with the control group MCV, HCT, MO% and MO were significantly decreased when compared with the compared with the control group In females, PCT was significantly increased in the 3 0g/kg group when compared with the control Most of those parameters were returned to normal except to HCT, PLT and MO during recovery period

In female, HCT and MCV in high dose group were significantly decreased MCH in low dose group, and RDW in high dose group were increased significantly, but no dose-response relationship was noted
All of those parameters were returned to normal during recovery period

Clinical chemistry (Table 13, 14, 15, 16)
In male, ALB value was significantly decreased in dosing group, and Crea value was significantly increased in low dose group, but no dose-response relationship was noted During recovery period, ALB in dosing groups and TG in low dose group were still higher than control

Other serum chemical values both in males or females did not differ between the control and dosing groups

Organ weights (Figure 5, 6, 7, 8 Table 5, 6, 7, 8)
No differences in treatment-related relative organ weights, i e the organ weight as a percentage of the body weight were observed during treatment and recovery periods Although relative heart, liver, lung weight of female rats in low dose group was higher than control, no dose-response relationship was observed

Macroscopic findings
No treatment related findings were recorded

Microscopic findings
No treatment related findings were recorded No histopathological changes were seen in the livers of 3 0g/kg animals on day 90 of the dosage regimen

No pathological changes were observed in treatment and control groups at the end of treatment period and 14 days recovery period

CONCLUSION

No treatment related effects were seen in rats after ninety days of oral treatment with Danggui Capsule at concentrations of 0 6 or 3 0 g/kg body weight/day and fourteen days recovery period

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Table 1 Body weight changes – 90-day Female Rats (week 0 to week 13)

Dose (g/kg)	0	0 6	3 0
No of animals	10	10	10
Week 0	190 50±7 37	191 70±7 32	189 30±6 77
Week 1	219 50±12 84	220 80±8 07	215 80±9 52
Week 2	237 70±14 24	241 20±12 70	235 70±10 86
Week 3	252 70±17 44	254 60±16 26	249 60±16 83
Week 4	267 00±20 60	264 70±14 20	260 00±19 56
Week 5	276 60±22 90	272 90±16 68	270 60±17 41
Week 6	283 10±25 19	284 90±18 50	278 50±16 11
Week 7	292 50±27 81	288 20±17 81	285 30±17 69
Week 8	301 70±27 23	291 60±18 49	287 10±18 71
Week 9	309 80±30 67	295 80±19 88	294 40±19 32
Week 10	298 40±30 00	296 80±19 08	298 60±15 90
Week 11	309 10±28 17	304 10±18 98	300 00±18 26
Week 12	313 50±30 65	302 10±23 37	312 60±21 81
Week 13	315 20±28 15	305 80±25 30	308 60±23 65

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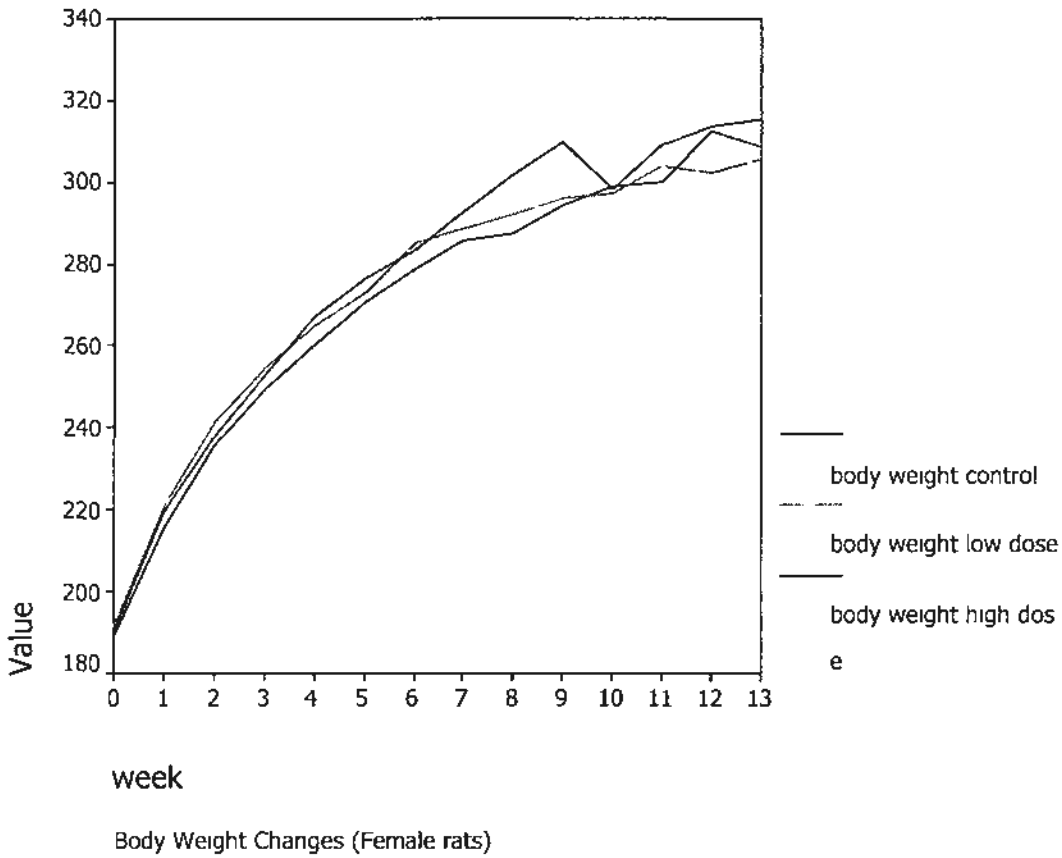

Figure 1 **Figure of Body Weight -- Female Rats (week 0 to week 13)**

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Table 2 Body weight changes – 90-day Male Rats (week 0 to week 13)

Dose (g/kg)	0	0 6	3 0
No of animals	10	10	10
Week 0	200 10±8 41	201 70±8 29	198 20±5 71
Week 1	274 00±13 47	275 80±8 16	276 90±6 10
Week 2	326 70±19 80	333 40±8 42	326 90±14 37
Week 3	366 40±25 03	383 10±10 87	365 20±14 76
Week 4	399 80±30 09	419 70±17 01	395 40±20 97
Week 5	427 10±34 94	453 40±21 98	425 00±26 95
Week 6	458 10±39 41	484 50±25 95	451 40±28 34
Week 7	481 60±41 99	511 20±26 54	472 60±30 60
Week 8	503 10±46 20	526 30±36 80	485 10±32 67
Week 9	524 10±52 42	545 80±37 73	503 00±38 19
Week 10	538 90±55 66	560 00±38 20	512 90±38 23
Week 11	553 10±55 77	578 90±41 87	526 40±42 66
Week 12	569 10±57 54	596 30±41 72	548 40±41 94
Week 13	575 30±58 92	601 90±41 63	545 70±34 29

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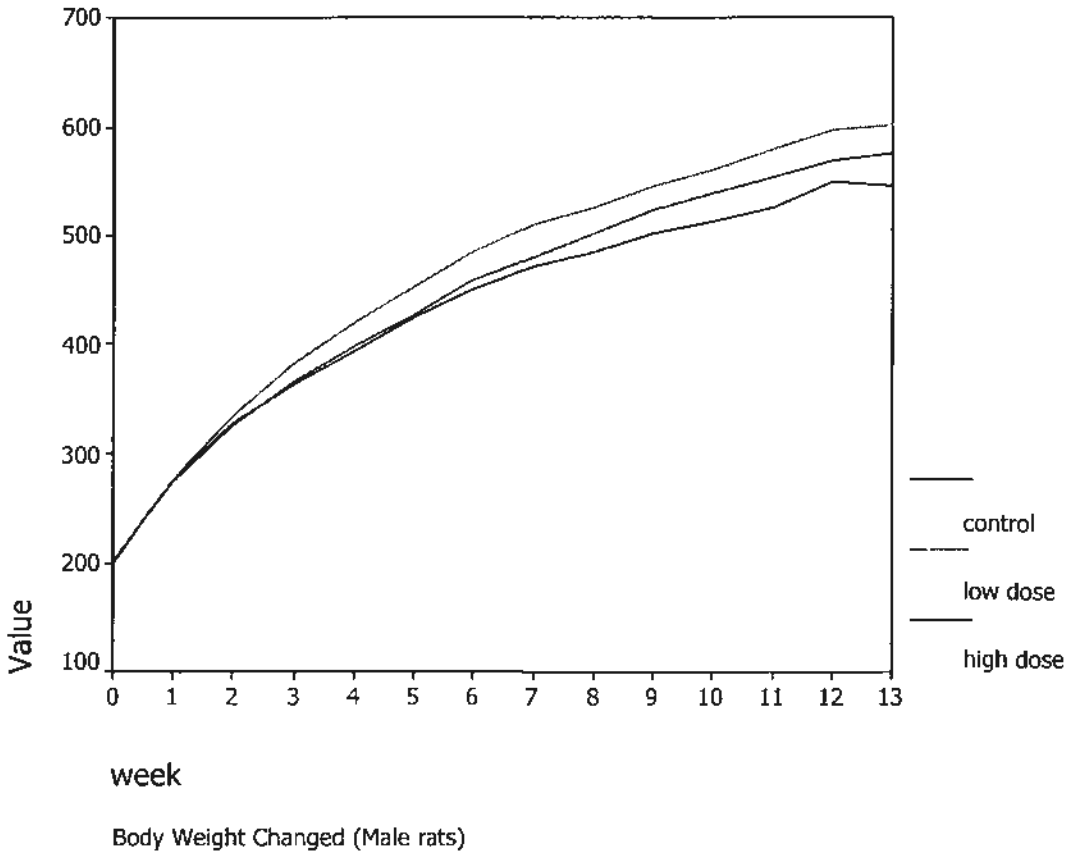

Figure 2 **Figure of Body Weight -- Male Rats (week 0 to week 13)**

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Table 3 Body weight changes – Recovery period Female Rats (week 14 and week 15)

Dose (g/kg)	0	0.6	3.0
No. of animals	3	3	3
Week 14	310.7 ± 17.1	306.7 ± 28.9	337.3 ± 8.33
Week 15	318.3 ± 16.9	308.0 ± 25.5	338.3 ± 3.1

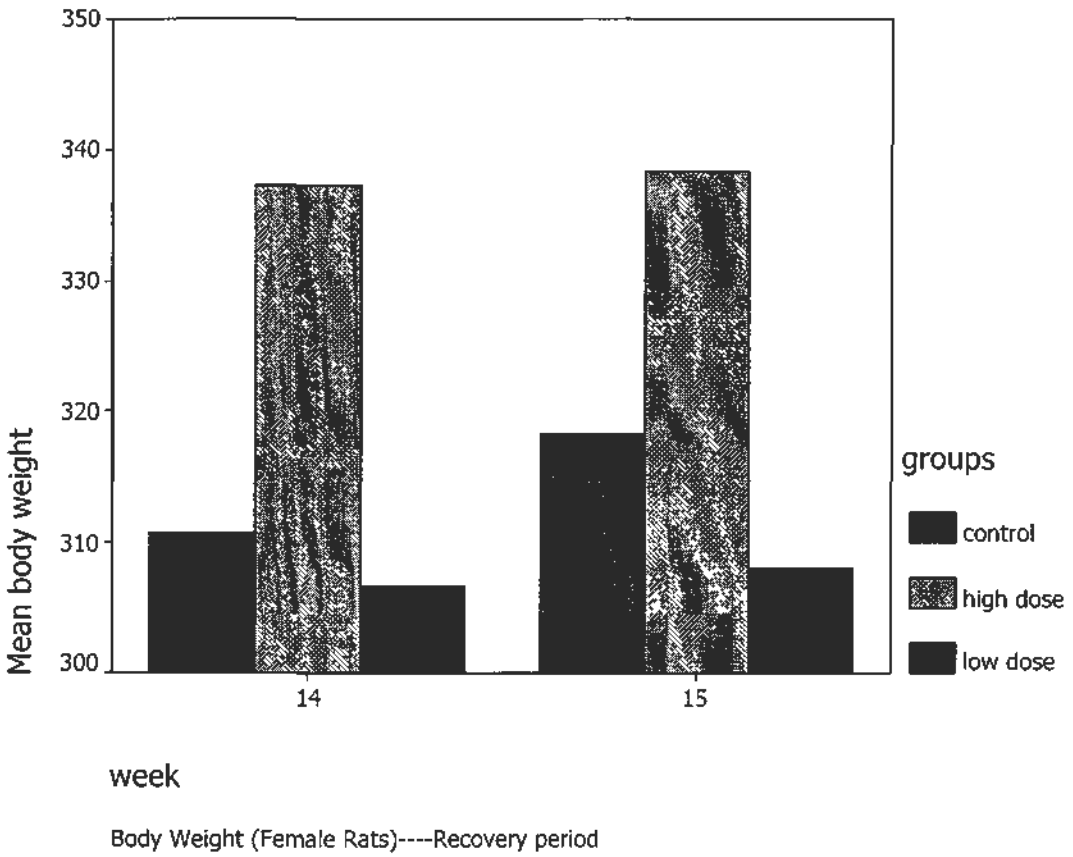

Figure 3 Figure of Female Rat Body Weight Changes – Recovery Period (Day 7 and day 14)

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Table 4 Body weight changes – Recovery period Male Rats (Week 14 and Week 15)

Dose (g/kg)	0	0.6	3.0
No. of animals	3	3	3
Week 14	496.0 ± 132.5	628.3 ± 37.0	503.0 ± 26.0
Week 15	494.3 ± 125.0	638.3 ± 36.4	516.3 ± 26.8

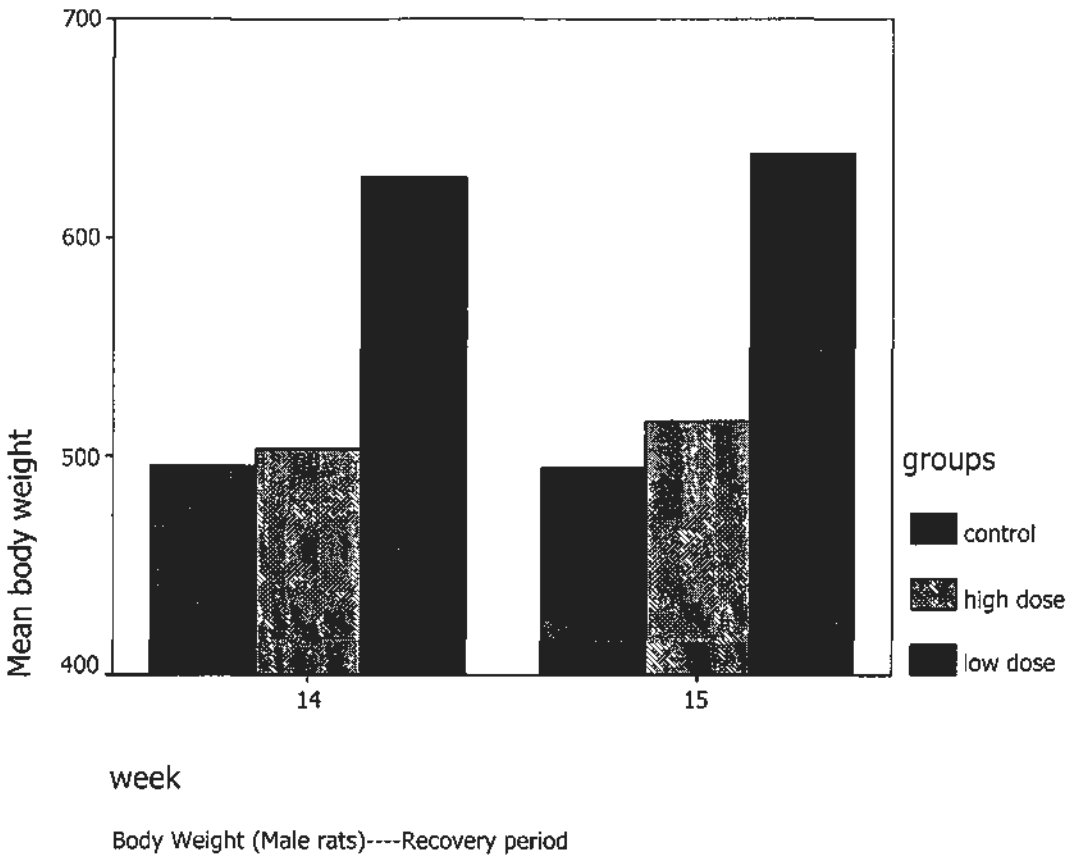

Figure 4 Figure of Male Rat Body Weight Changes – Recovery Period (Week 14 and Week 15)

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Table 5 Relative organ weight (% of body weight) of Female rats treated orally with Danggui Capsule for 90 days

Dose (ml/day)	0	0.6	3.0
No. of animals	10	10	10
Heart	0.30±0.02	0.32±0.03*	0.31±0.02
Liver	2.95±0.16	3.20±0.61	3.13±0.45
Spleen	0.17±0.03	0.18±0.03	0.16±0.03
Lung	0.50±0.05	0.50±0.09	0.43±0.07
Kidney	0.63±0.03	0.63±0.04	0.64±0.05

*p<0.05 compared with control group

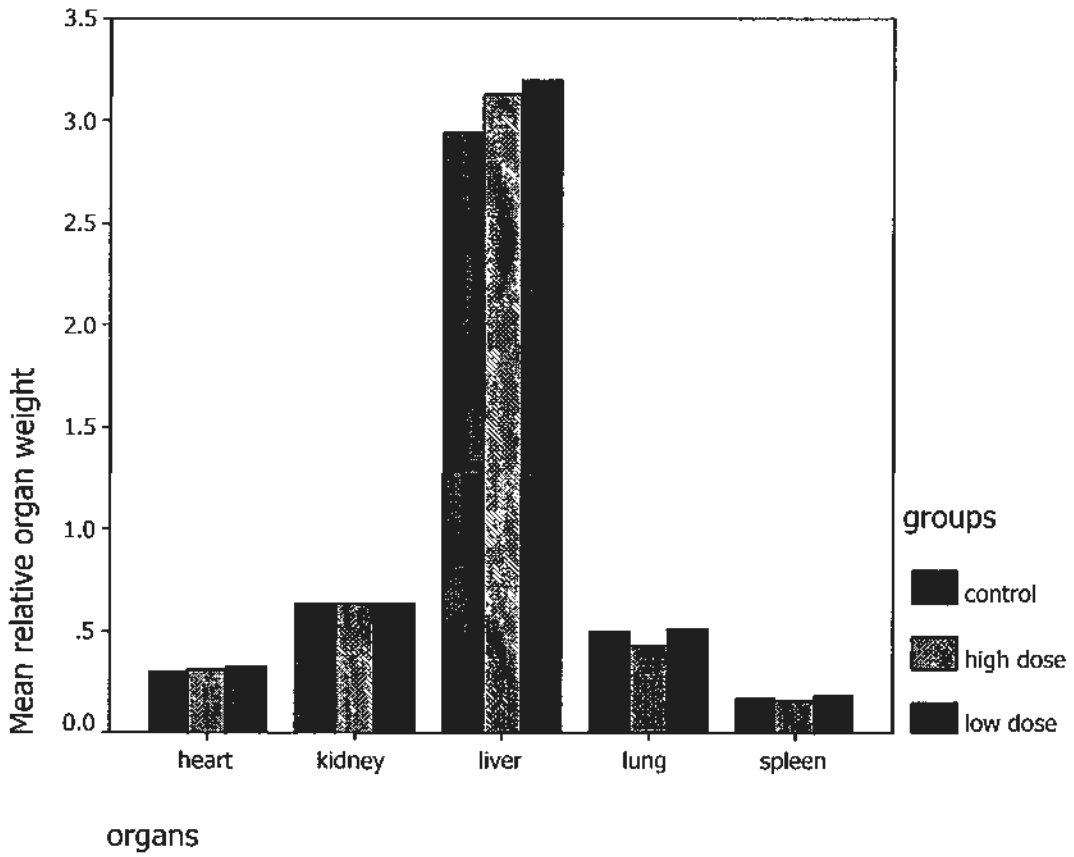

Figure 5 Figure of Relative Organ Weights of Female Rats

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Table 6 Relative organ weight (% of body weight) of Male rats treated orally with Danggui Capsule for 90 days

Dose (ml/day)	0	0.6	3.0
No. of animals	10	10	10
Heart	0.68±0.03	0.69±0.02	0.69±0.02
Liver	3.48±0.36	3.22±0.17	3.33±0.65
Spleen	0.15±0.02	0.14±0.01	0.14±0.02
Lung	0.38±0.09	0.35±0.08	0.36±0.05
Kidney	0.66±0.05	0.61±0.06	0.63±0.05

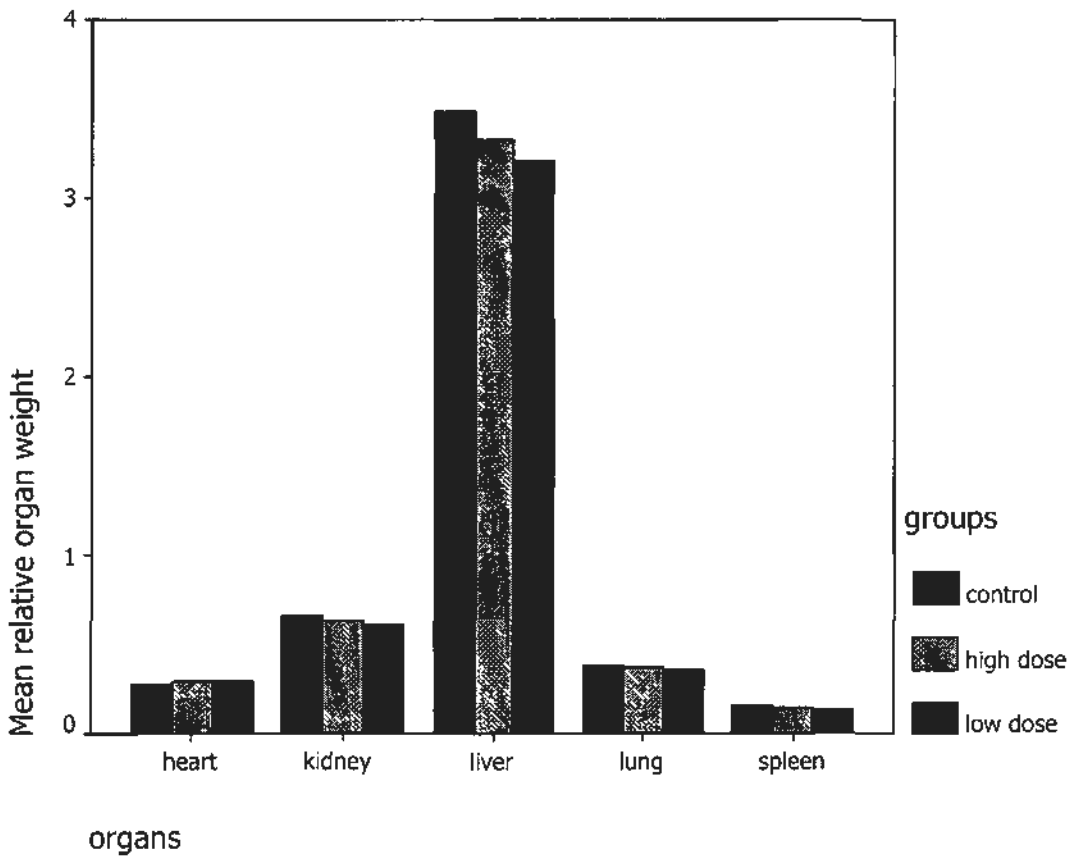

Figure 6 **Figure of Relative Organ Weights of Male Rates**

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Table 7 Relative organ weight (% of body weight) of Female rats treated orally with Danggui Capsule for 90 days—Recovery Period

Dose (ml/day)	0	0.6	3.0
No. of animals	3	3	3
Heart	0.31±0.01	0.32±0.01	0.67±0.01*
Liver	3.27±0.14	3.45±0.08	3.37±0.68
Spleen	0.19±0.02	0.19±0.01	0.19±0.02
Lung	0.39±0.06	0.36±0.01	0.36±0.01
Kidney	0.62±0.02	0.65±0 02	0.58±0.09

* p<0.05, compared with control

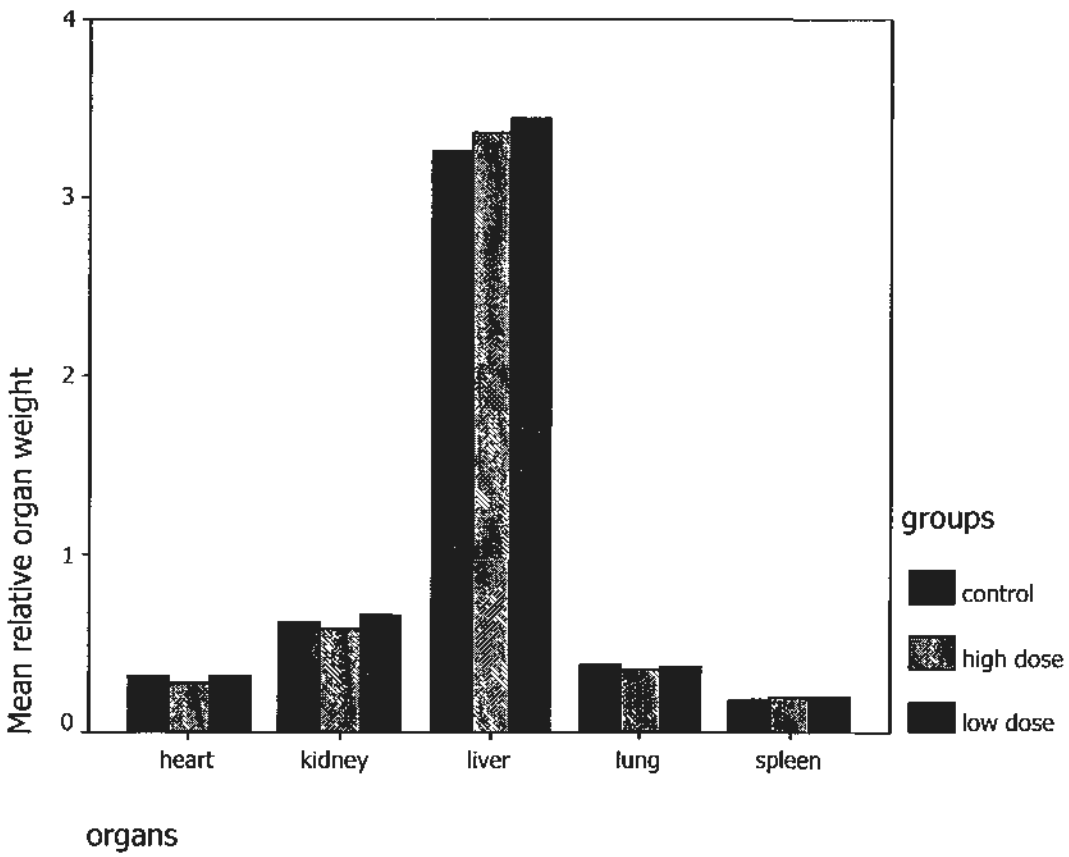

Figure 7 Relative organ weight of female rats---Recovery Period

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Table 8 Relative organ weight (% of body weight) of Male rats treated orally with Danggui Capsule for 90 days—Recovery Period

Dose (ml/day)	0	0.6	3.0
No. of animals	3	3	3
Heart	0.30±0.08	0.63±0.01	0.66±0.01
Liver	2.94±0.80	2.43±0.04	2.96±0.08
Spleen	0.17±0.02	0.17±0.02	0.14±0.01
Lung	0.42±0.06	0.37±0.01	0.39±0.02
Kidney	0.65±0.16	0.53±0.02	0.67±0.04

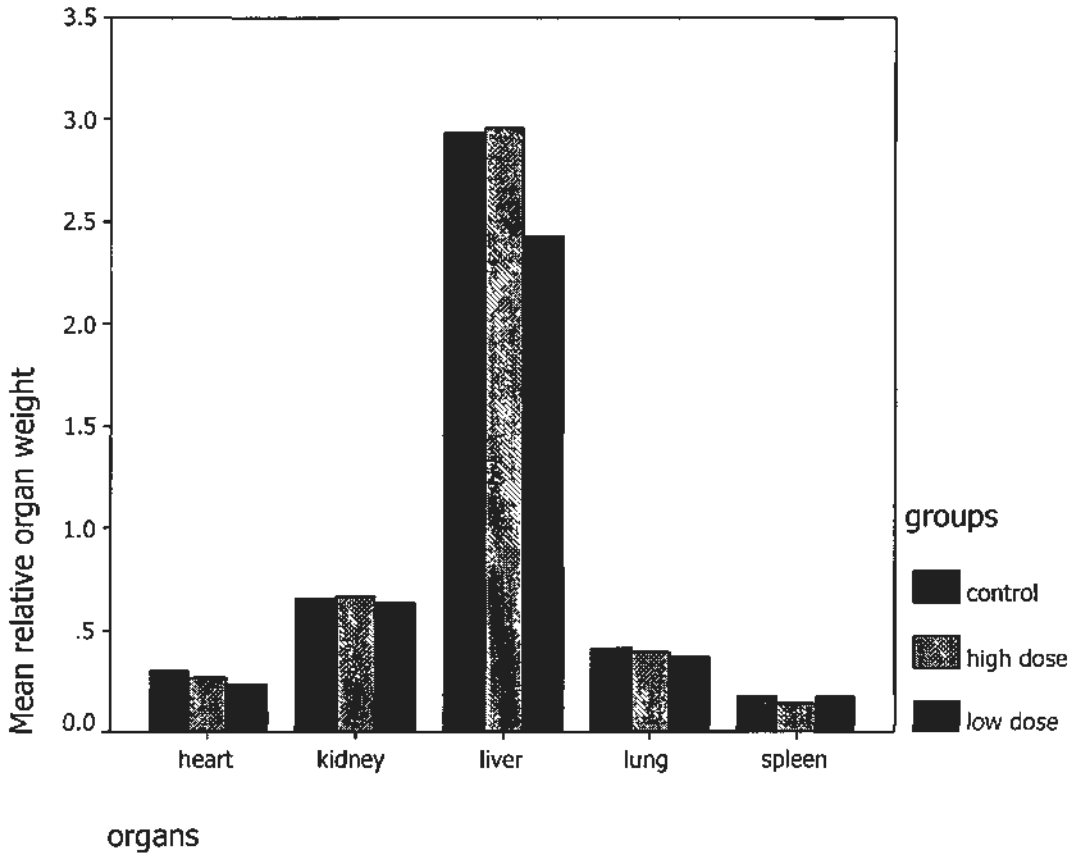

Figure 8 Relative organ weight of male rats---Recovery Period

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Table 9 Haematological findings of Male rats treated orally with Danggui Capsule for 90 days

Dose (ml/day)	0	0 6	3 0
No of animals	10	10	10
WBC	18 64±5 41	18 25±3 77	19 54±4 69
RBC	8 66±0 45	8 26±0 51	8 32±0 59
Hb	158 00±7 54	155 50±5 95	157 20±5 29
HCT	51 69±2 99	47 71±2 43*	47 57±2 23*
MCV	59 67±1 49	57 81±2 11*	57 27±2 51*
MCH	18 25±0 61	18 86±0 85	18 93±1 06
MCHC	306 00±9 07	326 20±8 53*	330 60±8 25*
LY%	85 74±8 90	77 21±9 84	77 83±12 87
MO%	4 02±0 64	2 95±0 59*	2 89±0 86*
GR%	10 64±8 51	19 84±9 78	19 28±13 38
PLT	253 50±34 99	297 00±53 02*	292 60±34 90*
LY	15 87±4 72	14 08±3 51	15 07±3 98
MO	0 77±0 63	0 55±0 19*	0 58±0 60*
GR	2 00±1 80	3 62±2 31	3 89±3 50
RDW	12 57±0 62	12 33±0 64	11 94±0 52*
PCT	0 17±0 02	0 61±0 04*	0 61±0 03*
MPV	6 95±0 64	7 14±0 19	7 14±0 34
PDW	13 52±0 58	12 97±0 60	13 08±0 54

* p<0 05 compared with control group

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Table 10 Haematological findings of Male rats -- Recovery period

Dose (ml/day)	0	0 6	3 0
No of animals	3	3	3
WBC	17 00±4 40	20 17±3 04	21 93±4 52
RBC	8 55±0 77	7 77±0 47	8 17±0 51
Hb	150 33±3 21	153 67±3 79	159 33±1 53
HCT	53 03±1 88	46 17±1 75*	48 07±2 26*
MCV	59 20±0 75	59 23±2 32	58 73±1 23
MCH	18 97±0 59	19 80±0 61	19 37±1 36
MCHC	410 33±162 80	334 67±9 29	333 67±13 20
LY%	344 20±71 02	79 93±9 39	69 50±12 00
MO%	3 33±0 50	3 30±0 36	3 00±0 96
GR%	9 00±6 68	27 30±11 69	24 47±21 47
PLT	248 00±21 66	321 67±32 75*	296 00±41 07
LY	16 20±7 39	13 80±3 31	15 37±2 95
MO	0 40±0 10	0 70±0 10*	0 59±0 17
GR	3 13±3 04	5 77±6 11	3 68±3 23
RDW	12 23±1 26	12 33±0 42	12 39±0 58
PCT	0 60±0 04	0 60±0 04	0 60±0 03
MPV	6 90±0 10	7 13±0 06	7 20±0 10
PDW	13 43±0 31	12 87±0 69	13 20±0 46

* p< 0 05 compared with control group

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Table 11 Haematological findings of Femal rats treated orally with Danggui Capsule for 90 days

Dose (ml/day)	0	0 6	3 0
No of animals	10	10	10
WBC	16 66±4 23	16 51±4 25	14 43±3 80
RBC	8 06±0 35	7 85±0 51	7 95±0 40
Hb	155 30±8 19	155 90±6 94	153 90±7 77
HCT	48 47±2 79	46 51±2 66	46 27±2 02*
MCV	60 15±1 77	59 31±1 05	58 25±2 00*
MCH	19 28±0 58	19 89±0 54*	19 37±0 68
MCHC	320 60±6 67	335 40±7 03*	332 50±10 75*
LY%	85 32±6 35	84 50±13 62	85 23±9 44
MO%	3 45±0 73	2 64±0 66*	3 22±1 22
GR%	11 23±6 21	12 86±13 78	11 55±8 44
PLT	247 20±40 69	274 10±43 56	266 30±56 18
LY	14 36±4 50	13 86±4 09	12 17±2 91
MO	0 58±0 19	0 47±0 18	0 49±0 31
GR	1 72±0 93	2 18±2 64	1 77±1 65
RDW	11 75±0 32	11 90±0 34	12 19±0 39*
PCT	0 17±0 04	0 19±0 03	0 19±0 05
MPV	6 88±0 69	7 13±0 66	7 18±0 32*
PDW	13 39±0 68	13 59±0 56	13 67±0 53

* p< 0 05 compared with control group

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Table 12 Haematological findings of Female rats -- Recovery period

Dose (ml/day)	0	0 6	3 0
No of animals	3	3	3
WBC	13 47±3 02	16 40±4 86	14 53±3 81
RBC	8 21±0 59	7 84±0 66	7 86±0 31
Hb	159 33±13 65	157 33±5 69	156 00±9 85
HCT	49 07±4 97	46 70±1 73	47 20±3 03
MCV	59 67±2 01	59 60±0 78	60 60±1 05
MCH	19 33±0 61	19 90±0 50	19 63±0 81
MCHC	325 00±8 00	336 33±9 24	327 33±16 17
LY%	79 40±4 16	88 00±7 99	86 67±2 23
MO%	3 60±1 08	3 33±0 12	3 30±0 66
GR%	17 27±3 36	8 63±7 94	10 07±1 88
PLT	233 67±50 05	273 00±65 05	230 00±59 77
LY	10 73±0 81	14 07±4 63	12 47±3 07
MO	0 50±0 10	0 57±0 15	0 60±0 17
GR	2 33±0 50	1 27±1 36	1 40±0 53
RDW	11 53±0 63	11 87±0 46	11 87±0 32
PCT	0 18±0 03	0 19±0 06	0 16±0 05
MPV	6 83±0 47	7 20±0 66	7 17±0 63
PDW	13 39±0 68	13 60±0 66	13 90±0 98

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Table 13 Clinical Chemistry of Male rats treated orally with Danggui Capsule for 90 days

Dose (ml/day)	0	0 6	3 0
No of animals	10	10	10
ALT	36 40±7 72	38 50±10 36	39 60±6 90
AST	111 00±22 80	107 60±17 29	114 70±17 08
GLU	6 64±0 95	6 76±0 35	6 75±0 57
TP	63 52±2 87	64 61±1 39	63 83±2 65
ALB	31 22±2 06	34 08±0 72*	33 77±1 21*
Crea	76 32±7 15	87 17±4 82*	81 55±8 80
BUN	5 65±0 85	6 16±0 68	5 59±3 02
CHOL	1 56±0 44	1 72±0 62	1 77±0 08
TG	1 01±0 34	1 31±0 30	1 03±0 68

* p< 0 05 compared with control group

Table 14 Clinical Chemistry of Male rats Recovery Period

Dose (ml/day)	0	0 6	3 0
No of animals	3	3	3
ALT	45 33±3 79	47 67±8 08	35 67±1 53
AST	118 67±18 01	101 67±14 84	110 00±22 07
GLU	7 47±0 38	6 92±0 37	6 94±0 67
TP	64 80±2 70	64 87±1 21	65 97±1 59
ALB	30 97±2 10	34 43±0 68*	34 83±3 07*
Crea	79 07±6 67	87 47±5 24	85 70±7 86
BUN	5 61±3 01	6 12±3 01	5 35±1 53
CHOL	1 97±0 55	1 83±0 13	1 77±0 06
TG	0 95±0 41	1 56±0 17*	1 03±0 41

* p< 0 05 compared with control group

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Table 15 Clinical Chemistry of Female rats treated orally with Danggui Capsule for 90 days

Dose (ml/day)	0	0 6	3 0
No of animals	10	10	10
ALT	37 5±9 08	41 50±8 24	34 30±12 24
AST	111 20±12 49	117 00±14 17	119 60±22 54
GLU	6 72±0 38	6 13±0 51*	7 02±0 37
TP	66 88±3 29	67 00±3 67	67 57±3 27
ALB	37 07±2 25	36 76±2 17	37 49±2 03
Crea	84 24±3 70	86 94±6 04	81 05±5 53
BUN	5 46±0 46	5 40±3 00	5 36±0 98
CHOL	2 04±0 34	2 05±0 66	2 00±0 65
TG	0 62±0 65	0 60±0 32	0 51±0 60

* p< 0 05 compared with control group

Table 16 Clinical Chemistry of Female rats Recovery Period

Dose (ml/day)	0	0 6	3 0
No of animals	3	3	3
ALT	35 00±2 65	43 33±9 50	39 67±8 02
AST	120 67±12 50	125 33±18 61	120 33±11 06
GLU	6 38±0 31	5 94±0 76	6 77±0 39
TP	64 50±3 86	67 57±5 77	68 57±1 72
ALB	35 77±3 76	37 70±2 61	38 57±1 78
Crea	83 53±3 11	87 17±2 83	84 50±4 25
BUN	5 29±0 04	5 13±0 76	5 24±0 79
CHOL	2 08±0 40	2 01±0 13	2 01±0 66
TG	0 58±0 64	0 80±0 48	0 53±0 62

Table 17 Histopathological findings in male rats treated with Danggui Capsule for 13 weeks

Dose (ml/day)	0										30									
Animal No	1	2	3	4	5	6	7	8	9	10	81	82	83	84	85	86	87	88	89	90
Heart Focal necrosis	1	1	3	3	1	1	1	1	1	1	1	1	1	2	1	1	1	3	1	1
Liver Focal necrosis	1	1	3	1	1	1	2	3	1	2	1	2	1	1	2	2	1	1	1	1
Spleen	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lung	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kidney	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Testes	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 18 Histopathological findings in female rats treated with Danggui Capsule for 13 weeks

Dose (ml/day)	0										30									
Animal No	11	12	13	14	15	16	17	18	19	20	91	92	93	94	95	96	97	98	99	100
Heart Focal necrosis	1	1	+	+	1	1	1	1	+	1	1	1	1	1	1	1	1	1	1	1
Liver Focal necrosis	1	1	1	1	2	3	1	1	1	1	1	2	2	1	1	1	1	1	1	2
Spleen	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lung	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Kidney	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ovaries	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

*** END OF REPORT ***

Appendix 10

Statistical Analysis Plan for Danggui Buxue Tang (當歸補血湯)

Project title:

A Randomized, Double-Blind, Placebo Controlled Study of the Effect of Danggui Buxue Tang (當歸補血湯) on Menopausal Symptoms and Quality of Life in Hong Kong Chinese Women

1 Objectives and Endpoints of the study

The primary objective of the study is to examine the effect of Danggui Buxue Tang (當歸補血湯, DBT) on menopausal symptoms of hot flushes and MENQOL. This study is also to evaluate the effect of Danggui Buxue Tang (當歸補血湯) on various risk markers for cardiovascular disease.

2 Endpoints

The primary endpoints:

The primary end-point of the study is the effect of treatment on the frequency of vasomotor symptoms

Change in severity and frequency of hot flushes and sweats

The second endpoints:

- Menopause Specific Quality of Life Questionnaire (MENQOL)
- Changes of various markers of risk for cardiovascular disease
- Vaginal maturation
- Arterial Reactivity

2 Group comparisons

Danggui Buxue Tang (當歸補血湯) treated group (Active Capsule group) vs. placebo treated group

3 Study design

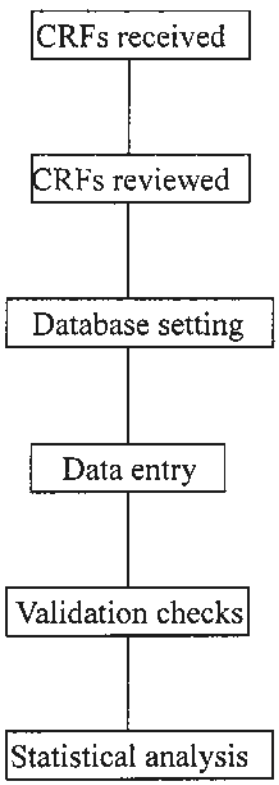
The study was designed as a single-center, randomized, double blind, placebo-controlled study. 100 healthy subjects were recruited and assigned to

receive Danggui Buxue Tang (當歸補血湯) (Active Capsule) or placebo in the 6 months study period.

4 Analysis Populations

- Population: Analyses are performed by complete treatment.
- Treated: Participants who never provided baseline & follow-up data are excluded.
- Per protocol: Participants who had major protocol violations are excluded.

5 Data management flow chart



6 Data Entry and Data Quality Control

The clinical data will be entered onto computer by means of Single Data Entry, which is made by a specialist keyboard operator. The data will be checked for completeness, accuracy and consistency. Any rogue or missing data will be highlighted and corrected before statistical analysis. The quality control checks include identifying any missing critical data, checking for consistency of responses and checking the completeness of dates and logical order.

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7 Analysis Plan

Database set up

Data from the research interviews and assessment instruments will be onto computer. The difference of variables between placebo group and active capsule group shall be compared using SPSS statistical package (version 11.5).

Demographic data

A summary of demographic data will be tabulation of the mean, minimum, maximum, standard deviation, and number of patients, for each quantitative measurement collected such as age, body weight, body mass index (BMI), blood pressure, pulse. Categorical data such as the numbers and percentages of patients in each of the categories will also be tabulated. These summaries will be presented overall, and separately for placebo and active capsule groups. (Table 1)

Age, blood pressure, heart rates and BMI in the DBT-treated and placebo-treated subjects will be compared using student *t*-test.

Vasomotor symptoms

Vasomotor symptoms will be analyzed as the frequency and severity of hot flushed as recorded on patient daily diary cards. The mean daily number of hot flushes is calculated as sum of the number of hot flushes on each day.

To assess severity of hot flushes, a daily severity score, which used the weights assigned to mild, moderate and severe hot flushes, will be calculated as:

> [(number of mild flushes × 1) + (number of moderate flushes × 2) + (number of severe flushes × 3)] ÷ total number of hot flushes on that day

An analysis of covariance of mean numbers of hot flashes will be conducted for DBT versus placebo at baseline, visit 2 and visit 3. Comparisons within group between baseline and visit 2 and visit 3 will also conducted. (Table 2,3)

MENQOL

The absolute difference in scores between Visit 2, Visit 3 and baseline for

each domain of the MENQOL Questionnaire, will be calculated.
The change in score from baseline to final visit assessment for the MENQOL for each group will be compared.
Repeated analysis of variance will be used to analyze the effects of treatment within and between groups over the study period. (Table 4,5)

Serum Hormone Data

The placebo-treated and DBT-treated groups will be compared using paired *t*-test for within group changes, and associated confidence intervals if measurements are normally distributed. If the measurements have a non-normal distribution, Mann-Whitney tests (Wilcoxon rank sum W test) with confidence intervals will be used. (Table 6)
A 2-way analysis of variance for change between the DBT-treated and placebo-treated groups will used to compare the differences.
For those lipid parameters for which there are significant baseline differences, a 2-way analysis of covariance will be used.

Serum Hormone Levels and Hot Flash Scores Relationship

The relationship of different hormone parameters and hot flash score in the two groups will be analyzed using Pearson Correlations Analysis if the data is normally distributed or Spearman Correlations Analysis if the data non-normal distribution.

Serum Lipid Data

Baseline and post-treatment lipid parameters will be compared using a paired *t*-test for within group changes, and a 2-way analysis of variance for change between the DBT-treated and placebo-treated groups.
A 2-way analysis of covariance will be used for those lipid parameters for which there are significant baseline differences.

Arterial Reactivity Data

Artery endothelial function for DBT-treated and placebo-treated groups will be compared using two-way analysis of variance, with group as a fixed factor and time (visit) as a repeated measures factor.
Simple linear regression will be used to assess the relationship between endothelium-dependent and –independent within the two groups.
The relationship between hot flash score and artery endothelial function variables will be analysis with the Pearson correlation test. (Table 7)

Cardiovascular Markers

Baseline measurements between groups will be compared using one-way analysis of variance.

Correlations between cardiovascular risk markers and vasomotor symptoms will be calculated with the Pearson or Spearman correlation coefficient.

Analysis of covariance (ANCOVA) for repeated measurements, with the baseline value of homocysteine as covariate, will be used for comparisons among and between the groups, and one-way analysis of variance will be used to test within-group changes. (Table 13)

Vaginal Maturation Index Data

Vaginal Maturation Index data will be analyzed within groups by the change from baseline using the Wilcoxon matched-pares signed-rank test and among groups using Wilcoxon's rank-sum test. (Table 14)

The maturation value (MV) is determined by 0.5 × fraction of intermediate cells + fraction of superficial cells × 1. (Table 15)

Safety data

Hematology and Biochemistry parameters at baseline and post-treatment will be compared using a paired t-test for within group changes, and unpaired t-tests will be used to compare both the baseline and the differences in the follow-up value between the two treatment groups. (Table 8)

Urinalysis data will be assessed using Chi-square test to compare the differences between the DBT-treated and Placebo-treated groups. (Table 9)

Adverse event data

Normally, it is rarely appropriate to use statistical significance tests to analyze safety data.

In the case of adverse event data, the rate of occurrence of each distinct adverse event on each treatment group will be reported, together with confidence intervals for the difference between rates of occurrence on the different treatments.

The results reflecting the changes and percent changes in safety parameters will be tested with Chi-squared test. (Table 10)

Compliance data

A summary of medication accounting, concurrent medications, and withdrawal/end-of-study data will be tabulated. (Table 11, 12)

8 Summary measurements

Quantitative variables will be described by standard descriptive statistics (mean, standard deviation, min and max) and qualitative variables will be summarized by frequency tables. Demographics and other baseline as well as treatment characteristics will be presented.

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Table 1 Demographic and Clinical Characteristics of Subjects

Baseline Characteristics	DBT	Placebo
Age at study entry (year)		
<55 years		
≥ 55 years		
Weight (kg)		
Body Mass Index (kg/m^2)		
≤25 kg/m^2		
> 25 kg/m^2		
Systolic Blood Pressure (mmHg)		
Diastolic Blood Pressure (mmHg)		
Heart Rate (beats/min)		
MENQOL domains		
Physical domain		
Vasomotor domain		
Psychosexual domain		
Sexual domain		

Table 2 Number of Hot Flushed per Month

Month	0*	1	2	3	4	5	6
DBT							
No. of subjects							
No. of hot flushed							
Placebo							
No. of subjects							
No. of hot flushed							

*0 = baseline

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Table 3. Severity of Hot Flushes

Month	0*	1	2	3	4	5	6
DBT							
No. of subjects							
No. of hot flushed							
Placebo							
No. of subjects							
No. of hot flushed							

*0 = baseline

Table 4. DBT compared with Placebo on MENQOL

	DBT				Placebo			
	Physical	Vasomotor	Psychosexual	Sexual	Physical	Vasomotor	Psychosexual	Sexual
Baseline								
Visit 2								
Visit 3								

Table 5. DBT compared with Placebo on MENQOL at Different Age and BMI groups

	DBT				Placebo			
	Physical	Vasomotor	Psychosexual	Sexual	Physical	Vasomotor	Psychosexual	Sexual
Age<55								
Age≥55								
BMI≤25								
BMI>25								

Table 6 FSH, LH and E2 levels at Baseline, Visit 2 and Visit 3 by Treatment of DBT and Placebo

	DBT			Placebo		
	Baseline	Visit 2	Visit 3	Baseline	Visit 2	Visit 3
FSH						
LH						
E2						

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Table 7 Artery Variables Comparison

	DBT			Placebo		
	Baseline	Visit 2	Visit 3	Baseline	Visit 2	Visit 3
Diameter (mm)						
Before						
After						
Absolute change in diameter (mm)						
Before						
After						
Percentage change in diameter (%)						
Before						
After						

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Table 8 Hematology and Biochemistry Comparison

	DBT			Placebo		
	Baseline	Visit 2	Visit 3	Baseline	Visit 2	Visit 3
Hemoglobin						
Erythrocytes						
White Blood Count						
Albumin						
Calcium						
Alkaline Phosphates						
Phosphatase						
Inorganic						
ALT/GPT						
Bilirubin						
Total Protein						
Creatinine						
Potassium						
Sodium						
Glucose						
Urea						

Table 9 Urinalysis data comparison at Baseline, Visit 2 and Visit 3

	DBT			Placebo		
	Protein	Glucose	Ketone	Protein	Glucose	Ketone
Baseline						
Negative						
Trace						
Positive						
Visit 2						
Negative						
Trace						
Positive						
Visit 3						
Negative						
Trace						
Positive						

Appendix 10

Table 10 Adverse Events during the Study

Adverse Event	DBT (N=)		Plasebo (N=)	
	No. of Women	%	No. of Women	%

Table 11 Reasons for Subject Dropout During the Study by Treatment Assignment

	No. of Subject	
Treatment violation	DBT	Placebo

Table 12 Compliance of Study Medication Taking

	DBT	Placebo
Total dose issued		
Returned dose		
Missing dose		
Compliance (%)		

Appendix 10

Table 13 Cardiovascular Risk Markers Concentrations

	DBT			Placebo		
	Baseline	Visit 2	Visit 3	Baseline	Visit 2	Visit 3
C-reactive protein						
Homocysteine						
Serotonin						
Interleukin-6						

Table 14 Vaginal Maturation

	Parabasal		Intermediate		Superficial	
	Baseline	Visit 3	Baseline	Visit 3	Baseline	Visit 3
DBT						
Placebo						

Table 15 Maturation Value (MV)

Group	Maturation Value (MV)	
	Baseline	Visit 3
DBT		
Placebo		

Randomized, Double-Blind, Placebo-Controlled Study to compare the Effect and Safety of a Herbal Capsule 壯骨膠囊 with placebo in Patients with Post-menopausal Osteoporosis

Sample size estimation

Sample size calculation will be based on a mean annual decrease in spinal BMD of approximately 1.9% in untreated women. The actual SD in our previous study was 3.6%. To detect a 1% difference in spinal BMD using a two-sided 0.05 α-level test with 90% power requires:

$$N = \left[\frac{(\mu_\alpha + \mu_\beta)\,\sigma}{\xi}\right]^2$$

N: Numbers of patients should be recruited

μ_α: 1.96 (two-side, α=0.05)

μ_β: 1.28 (power=0.90)

σ: 3.6%

ξ: 1%

$$N = \left[\frac{(1.96 + 1.28)\,0.036}{0.01}\right]^2 = 136$$

According to previous studies, approximately 10% of subjects remained the study free at the end of the trial. The total number of subjects will be:

$$N = \frac{136}{1 - 0.10} = 150$$

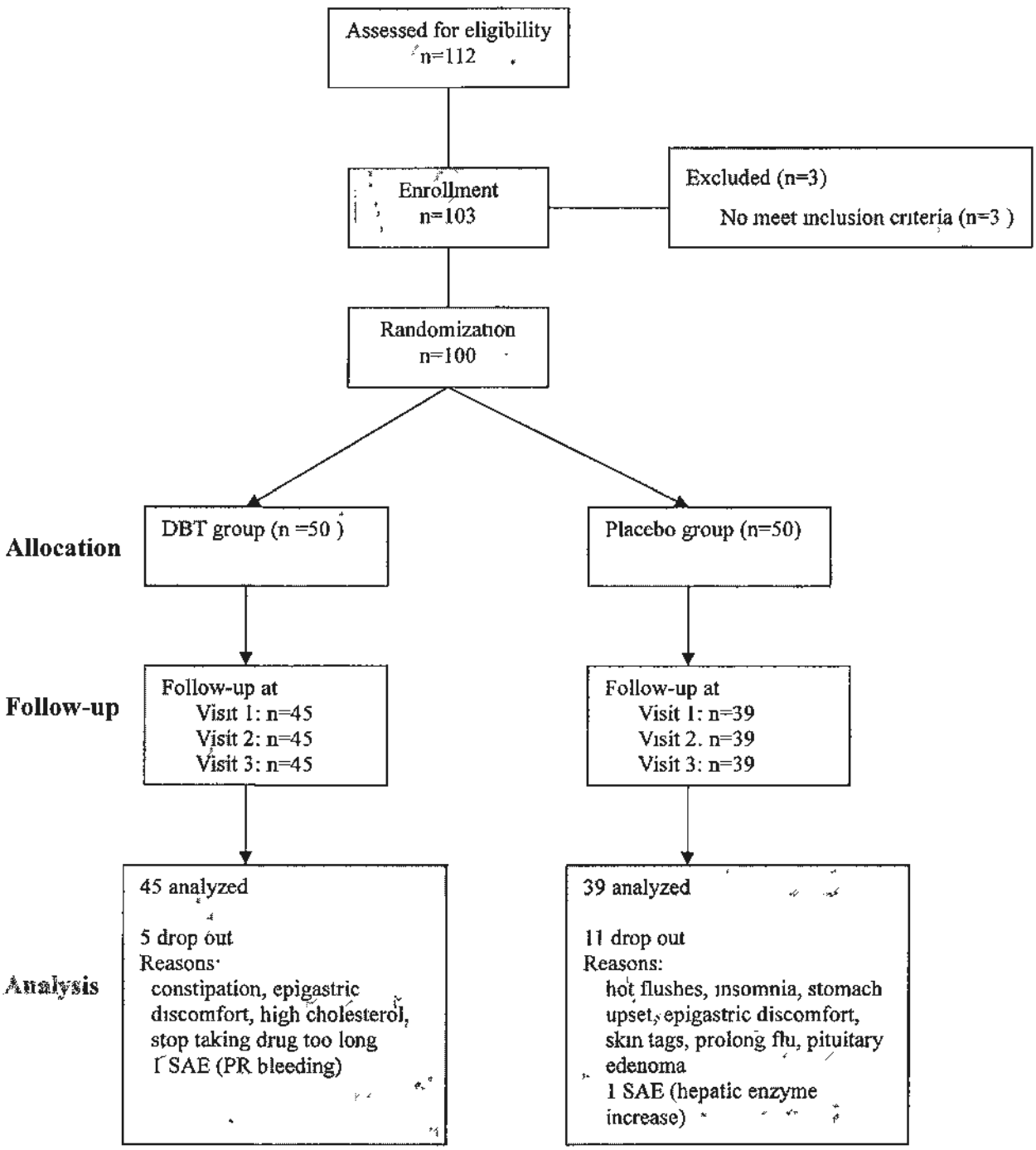
A sample size of 150 postmenopausal osteoporosis patients will be used. With this number, an at least 1% difference in BMD between the treatment with herbal formula capsules and placebo can be detected with 90% power if the standard deviation of BMD measurements is 3.6%.

Appendix 12

Statistical Analytical Report

Title: A randomized, double-blind, placebo-controlled study of the effect of Danggui Buxue Tong on menopausal symptoms and quality of life in Hong Kong Chinese women

Design and conduct of the trial:



Appendix 12

Parameters analyzed in the preliminary report

1 General information
2 Hot Flushes per month at baseline and during study treatment
3 Changes in MENQOL (four domains)
- Vasomotor symptoms analysis
  Hot Flushes
  Night Sweats
  Sweating
- Psychosocial
- Physical
- Sexual
4 VMI, superficial, intermediate, and parabasal cells analysis
5 Hormone levels analysis
6 Blood chemistry
7 Adverse events

Software used in the analysis: SPSS 11.5 for Windows

Statistical methods used for data analysis

1 General information
Baseline characteristics were summarized by treatment group.
For continuous variables, means were compared using analysis of variance.
Categorical variables were compared using the χ^2 test.

2 Hot flashes
Changes from baseline in the number and severity of hot flashes were assessed within treatment groups by using pared t-test. Comparisons to placebo were conducted using an ANCOVA wit baseline as a covariate.
The mean daily number and severity of hot flashes were compared among treatment groups for each month using an ANCOVA, with treatment as a factor in the model and baseline as the covariate.
Differences in categorical data between groups were explored by Mann-Whitney Test, Wilcoxon Signed Ranks Test or χ^2 test.
A linear regression analysis was used to analyze the relationship between duration of menopause and the decrease in hot flashes during treatment.

3 MENQOL
Changes in MENQOL scores from baseline to month 3 and month 6 were

analyzed with analysis of variance and analysis (ANOVA) of covariance (ANCOVA).

4 Vaginal maturation index (VMI)

VMI was analyzed by using a two-way of variance or Student's t-test or χ^2 test.

A two-way analysis of variance was used to analyze the maturation index; for the purposes of statistical analysis, a maturation index of 52 or less was used to define the population with atrophy.

5 Hormone levels analysis

Student's t-test and ANOVA were used to compare the differences of the Hormone levels.

6 Adverse events

χ^2 test was used to compare the incidence between the two treatment groups.

1 General information

Table 1 Baseline Demographic characteristics of the all-randomized population

	DBT	Placebo	DBT vs. Placebo P value	All Subjects
No. of patients	45	39		84
Age (y)				
Current	52.8±4.9	51.3±4.6	0.153	52.0±4.8
At menopause	47.1±5.8	46.1±4.5	0.356	46.6±5.2
Duration of menopause (y)	5.7±4.3	5.2±3.3	0.618	5.5±3.9
Body mass index (kg/m^2)	23.6±3.2	22.0±2.9	0.019	22.9±3.2
Body weight (kg)	57.6±9.0	53.4±7.0	0.019	55.6±8.4
Systolic blood pressure (mmHg)	124.3±17.5	118.9±16.1	0.156	121.8±17.0
Diastolic blood pressure (mmHg)	73.4±12.2	70.6±10.3	0.258	72.1±11.4
FSH	70.57±24.79	87.62±29.96	0.006	
LH	33.91±12.20	42.55±15.17	0.005	
E2	51.95±17.73	48.29±6.49	0.231	

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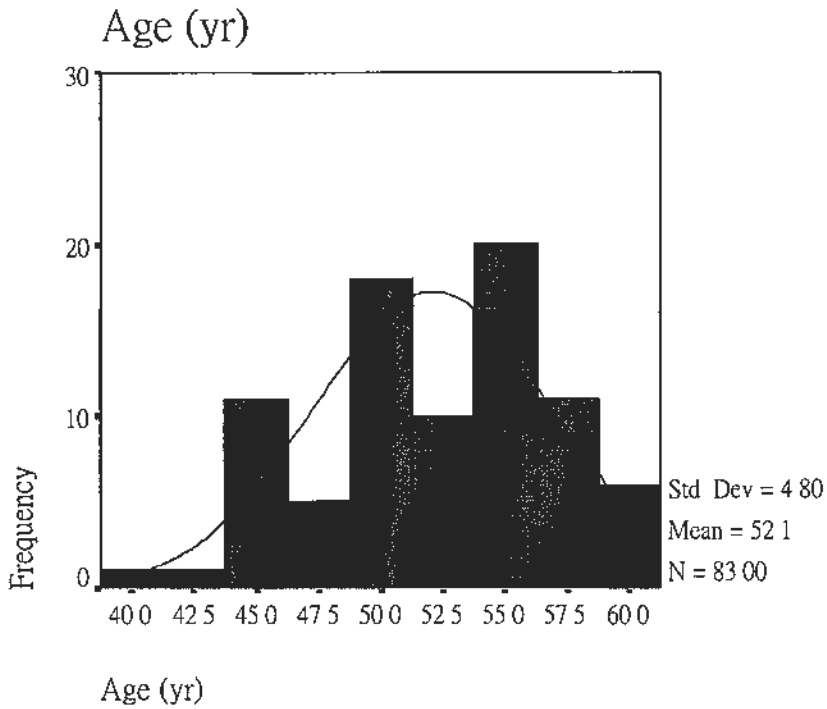

Age (yr)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	39	1	1 2	1 2	1 2
	43	1	1 2	1 2	2 4
	44	5	6 0	6 0	8 4
	45	5	6 0	6 0	14 5
	46	1	1 2	1 2	15 7
	47	2	2 4	2 4	18 1
	48	3	3 6	3 6	21 7
	49	5	6 0	6 0	27 7
	50	6	7 2	7 2	34 9
	51	7	8 4	8 4	43 4
	52	6	7 2	7 2	50 6
	53	4	4 8	4 8	55 4
	54	12	14 5	14 5	69 9
	55	4	4 8	4 8	74 7
	56	4	4 8	4 8	79 5
	57	5	6 0	6 0	85 5
	58	6	7 2	7 2	92 8
	59	2	2 4	2 4	95 2
	60	3	3 6	3 6	98 8
	61	1	1 2	1 2	100 0
	Total	83	100 0	100 0	

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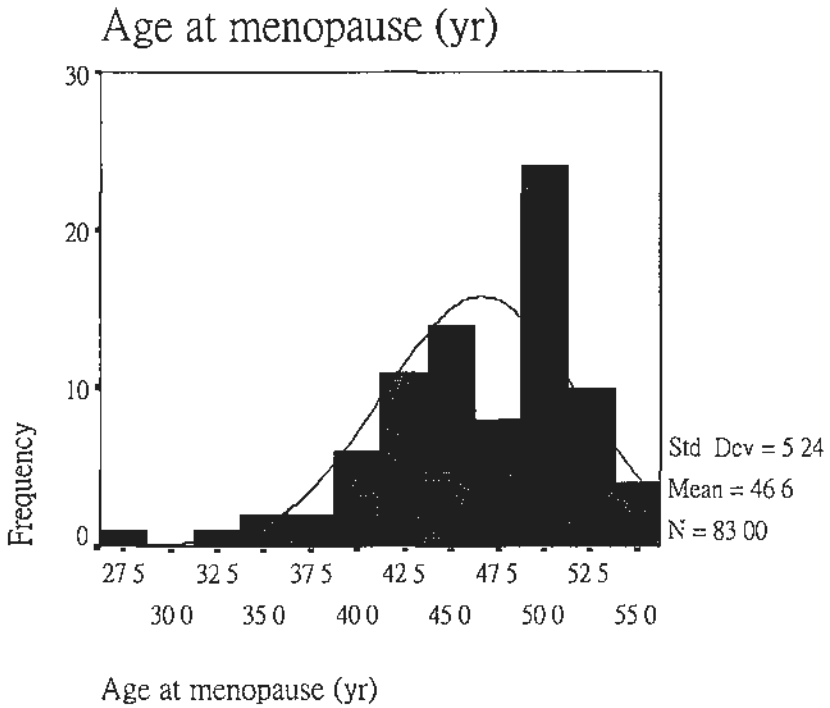

Age at menopause (yr)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	28	1	1 2	1 2	1 2
	33	1	1 2	1 2	2 4
	35	2	2 4	2 4	4 8
	38	2	2 4	2 4	7 2
	40	2	2 4	2 4	9 6
	41	4	4 8	4 8	14 5
	42	5	6 0	6 0	20 5
	43	6	7 2	7 2	27 7
	44	3	3 6	3 6	31 3
	45	7	8 4	8 4	39 8
	46	4	4 8	4 8	44 6
	47	4	4 8	4 8	49 4
	48	4	4 8	4 8	54 2
	49	10	12 0	12 0	66 3
	50	9	10 8	10 8	77 1
	51	5	6 0	6 0	83 1
	52	8	9 6	9 6	92 8
	53	2	2 4	2 4	95 2
	54	1	1 2	1 2	96 4
	55	2	2 4	2 4	98 8
	56	1	1 2	1 2	100 0
	Total	83	100 0	100 0	

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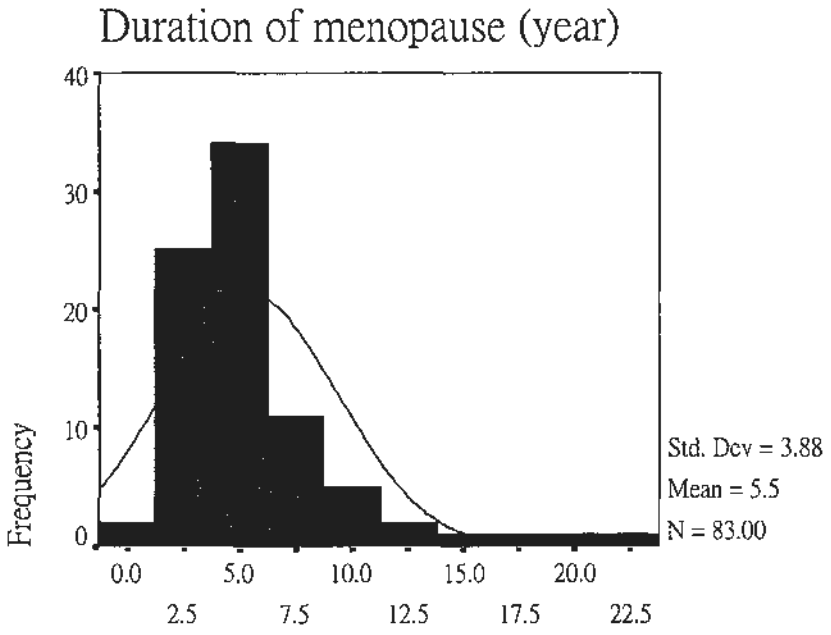

Duration of menopause (year)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1.0	2	2.4	2.4	2.4
	2.0	8	9.6	9.6	12.0
	3.0	17	20.5	20.5	32.5
	4.0	18	21.7	21.7	54.2
	5.0	11	13.3	13.3	67.5
	6.0	5	6.0	6.0	73.5
	7.0	7	8.4	8.4	81.9
	8.0	4	4.8	4.8	86.7
	9.0	3	3.6	3.6	90.4
	10.0	1	1.2	1.2	91.6
	11.0	1	1.2	1.2	92.8
	12.0	2	2.4	2.4	95.2
	15.0	1	1.2	1.2	96.4
	18.0	1	1.2	1.2	97.6
	19.0	1	1.2	1.2	98.8
	23.0	1	1.2	1.2	100.0
	Total	83	100.0	100.0	

Eighty-three subjects completed the clinical trial. The age range from 39 to 61 years, with a mean (± SD) of 52.1± 4.8 years. The mean age at menopause was 46.6 ± 5.24, with range of 28 to 56 years. Demographic characteristics of the groups are summarized in Table 1. The intervention and control groups were comparable at baseline except for Hormone levels (FSH and LH), which were higher in placebo

group (p=0.006 and 0.005).

2 Self-reported Data on Hot Flashes

Table 2 Number of Mild Hot Flushes each Month During Study Period

Month	1	2	3	4	5	6	P value Month 1 vs. Month 6
DBT							
< 4*	27±25	23±26	24±35	16±22	16±20	14±22 (-48%)	0.032
> 5*	10±19	8±13	5±11	6±11	4±8	2±5 (-80%)	0.008
All subjects	19±24	16±22	16±28	11±18	10±17	9±17 (-53%)	0.002
Placebo							
< 4*	29±44	18±27	18±26	19±25	17±25	14±19 (-52%)	0.135
> 5*	24±44	20±43	17±34	19±29	18±23	11±16 (-54%)	0.328
All subjects	27±44	19±35	18±30	19±27	17±24	12±18 (-56%)	0.062

* Menopause duration (years)

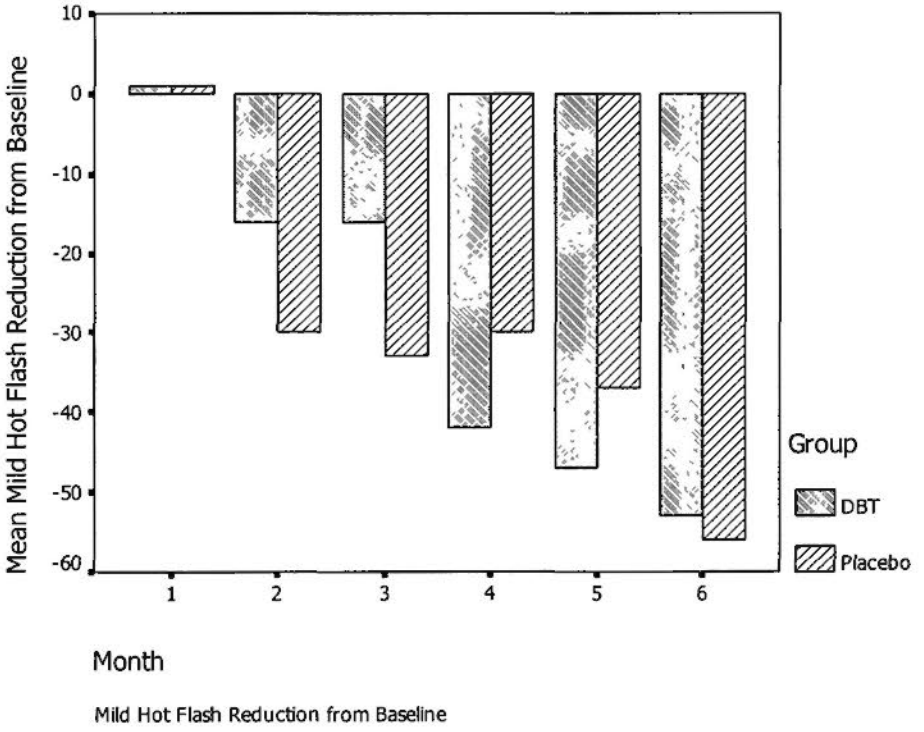

Figure 1 Hot Flashes Reduction from Baseline

Table 3 Mean number of hot flashes at baseline (1st week) and week 24

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	DBT (n=45)		Placebo (n=38)		P value
	Mean hot flash	95% CI	Mean hot flash	95% CI	DBT vs. placebo
Baseline (1st wk)	1.33	0.78, 1.88	1.99	1.27, 2.83	0.123
Week 24	0.50	0.23, 0.77	0.96	0.43, 1.52	0.105
%Reduction from baseline	62.4		51.8		
P value (wk 1 vs. wk 24)	0.001		0.002		

The baseline was defined as the mean of first seven days of hot flashes after administration of DBT or placebo.

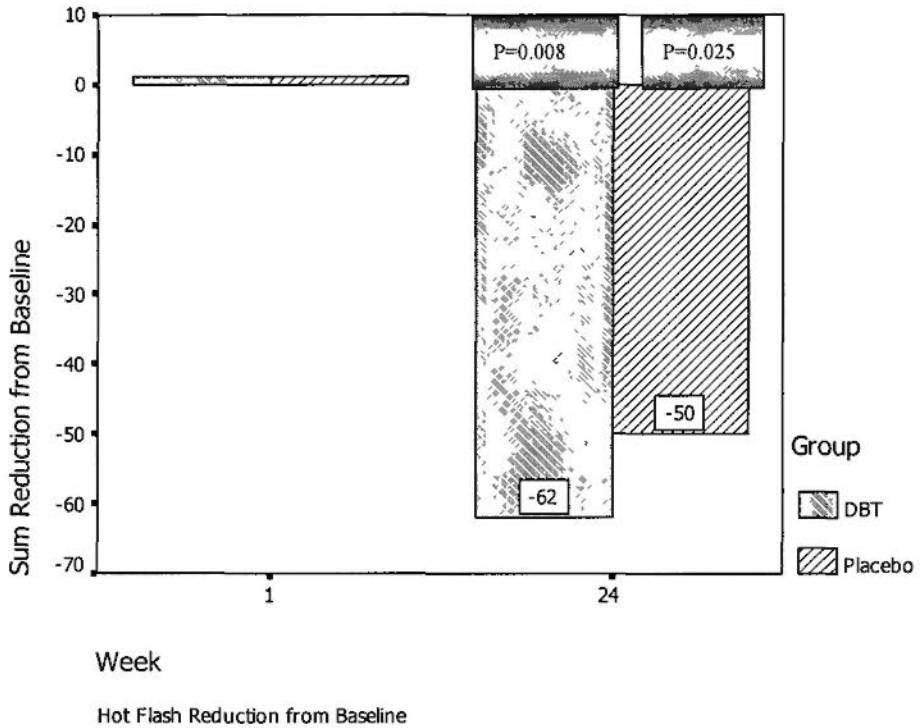

Figure 2 Mean hot flashes reduction at week 24 compared with week 1

Table 2 and 3 list mean of numbers of mild hot flashes and the overall reduction in means from baseline to completion of the study. The numbers of mild hot flashes in DBT group were significantly declined when compared with baseline (p=0.002). However the difference of mild hot flashes in Placebo group between baseline and the end of the study was not significant (p=0.062). The total number of hot flashes, including mild, moderate and severe, were dropped about 62% in DBT group (p=0.008); in Placebo group, about 50% decline were observed (p=0.025).

3 Score Changes of MENQOL

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Table 4 Mean Score of MENQOL at each Visit (N=83)

	DBT	Placebo	p-value DBT vs. Placebo
Vasomotor domain			
Visit 1			
Age < 50	2.83±2.04	2.69±1.66	
Age > 51	2.81±1.98	2.88±1.61	
All patients	2.82±1.97	2.81±1.60	0.978
Visit 2			
Age < 50	1.92±1.19	1.97±1.16	
Age > 51	2.27±1.35	1.89±1.10	
All patients	2.14±1.29	1.92±1.10	0.486
Visit 3			
Age < 50	2.53±1.58	1.72±1.47	
Age > 51	2.15±1.58	1.74±1.73	
All patients	2.29±1.56	1.73±1.31	0.149
P value	Age < 50: 0.466	Age < 50: 0.143	
(visit 3 vs. visit 1)	Age > 51: 0.168	Age > 51: 0.022	
	All patients: 0.247	All patients: 0.006	
Psychosocial domain			
Visit 1			
Age < 50	1.64±1.12	2.11±1.58	
Age > 51	1.91±1.70	1.64±1.73	
All patients	1.83±1.54	1.85±1.66	0.966
Visit 2			
Age < 50	1.83±0.90	1.76±1.33	
Age > 51	1.81±1.57	1.66±1.31	
All patients	1.82±1.41	1.71±1.29	0.733
Visit 3			
Age < 50	2.38±1.47	1.62±1.32	
Age > 51	1.99±1.40	1.49±1.39	
All patients	2.09±1.41	1.55±1.34	0.098
P value	Age < 50: 0.189	Age < 50: 0.360	
(visit 3 vs. visit 1)	Age > 51: 0.857	Age > 51: 0.778	
	All patients: 0.431	All patients: 0.421	
Physical domain			
Visit 1			
Age < 50	1.96±1.12	2.52±1.37	
Age > 51	2.54±1.38	1.76±1.21	
All patients	2.36±1.32	2.06±1.32	0.300
Visit 2			
Age < 50	2.04±1.25	2.03±1.12	
Age > 51	1.92±1.22	1.63±1.10	
All patients	1.96±1.22	1.79±1.11	0.536
Visit 3			
Age < 50	2.60±1.53	1.90±1.09	
Age > 51	2.11±1.40	1.51±0.94	
All patients	2.26±1.44	1.67±1.00	0.034
P value	Age < 50: 0.225	Age < 50: 0.178	
(visit 3 vs. visit 1)	Age > 51: 0.232	Age > 51: 0.457	
	All patients: 0.724	All patients: 0.154	
Sexual domain			
Visit 1			
Age < 50	3.79±2.13	3.42±1.92	
Age > 51	3.35±1.91	3.02±2.11	
All patients	3.49±1.96	3.20±2.00	0.569
Visit 2			
Age < 50	3.29±1.95	2.74±2.24	
Age > 51	2.21±1.52	2.79±1.92	
All patients	2.53±1.70	2.77±2.00	0.652

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Visit 3					
Age < 50	3.12±2.38		2.37±1.76		
Age > 51	2.51±1.38		2.88±2.25		
All patients	2.73±1.80		2.63±2.00		0.855
P value	Age < 50:	0.275	Age < 50:	0.106	
(visit 3 vs. visit 1)	Age > 51:	0.005	Age > 51:	0.388	
	All patients:	0.006	All patients:	0.069	

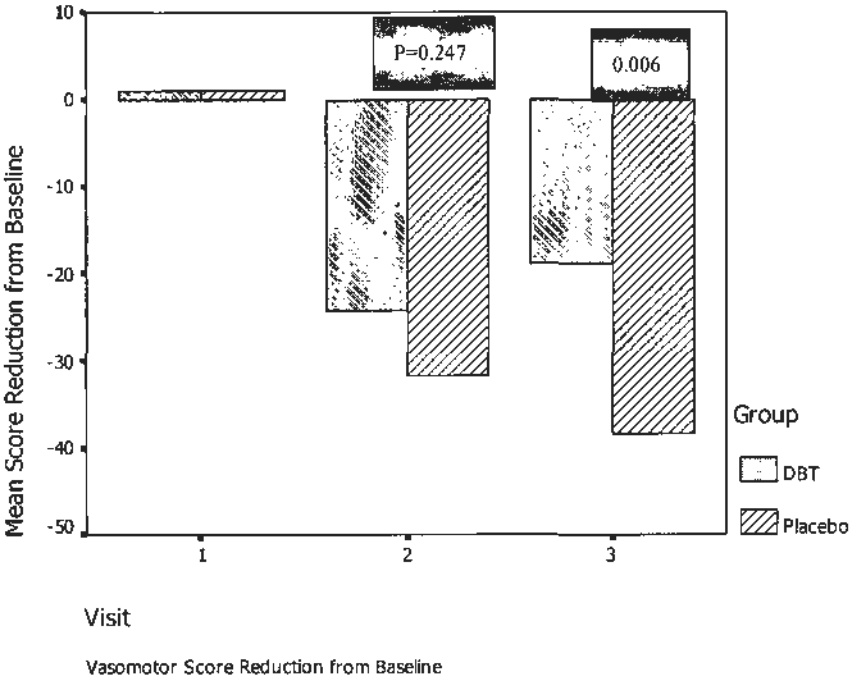

Figure 3 Score of Vasomotor Domain Reduction from Baseline

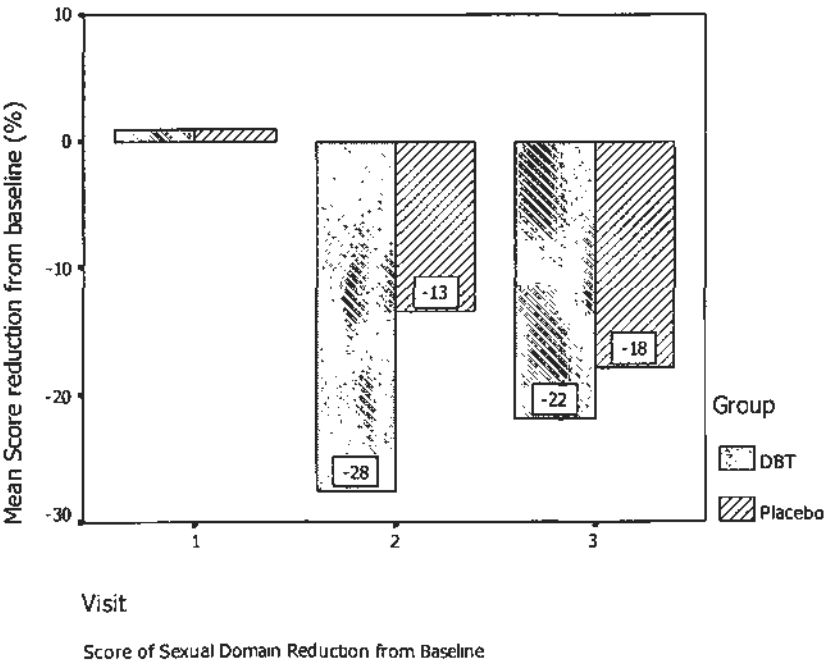

Figure 4 Score of Sexual Domain Reduction from Baseline

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Table 5 Incidence of Vasomotor symptoms (%)

Group	Visit 1	Visit 2	Visit 3	P value (visit 1 vs. Visit 3)
DBT	77.8	71.1	62.2	0.107
Placebo	81.6	76.3	78.9	0.773
P value (DBT vs. Placebo)	0.669	0.592	0.098	

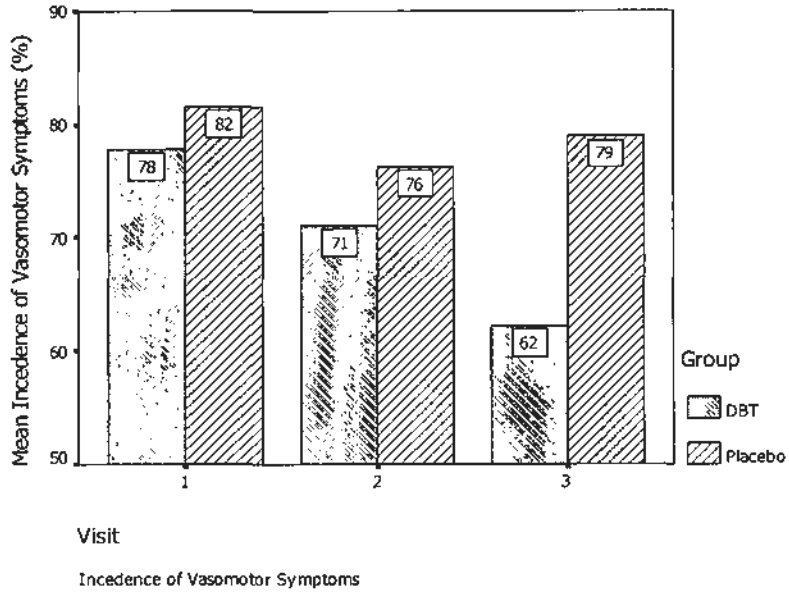

Figure 5 Incidence Comparisons in Vasomotor Symptoms

Table 10 Vasomotor Domain: Incidence comparison (N=83)

	DBT (n=45) (%)	Placebo (n=38) (%)	p-value (DBT vs. Placebo)
Hot Flushes			
Baseline (visit 1)	32	32	
Visit 2	24(-25.0)	27(-15.6)	
Visit 3	24(-25.0)	26(-18.8)	0.162
p-value			
Visit 1 vs. visit 2	0.082	0.169	
Visit 1 vs. visit 3	0.082	0.105	
Night Sweats			
Baseline (visit 1)	22	17	
Visit 2	17(-22.7)	15(-11.8)	
Visit 3	10(-54.5)	10(-41.2)	0.664
p-value			
Visit 1 vs. visit 2	0.288	0.093	
Visit 1 vs. visit 3	0.008	0.642	
Sweating			
Baseline (visit 1)	26	21	
Visit 2	24(-7.7)	19(-9.5)	
Visit 3	20(-23.1)	15(-28.6)	0.648
p-value			
Visit 1 vs. visit 2	0.671	0.646	
Visit 1 vs. visit 3	0.206	0.168	

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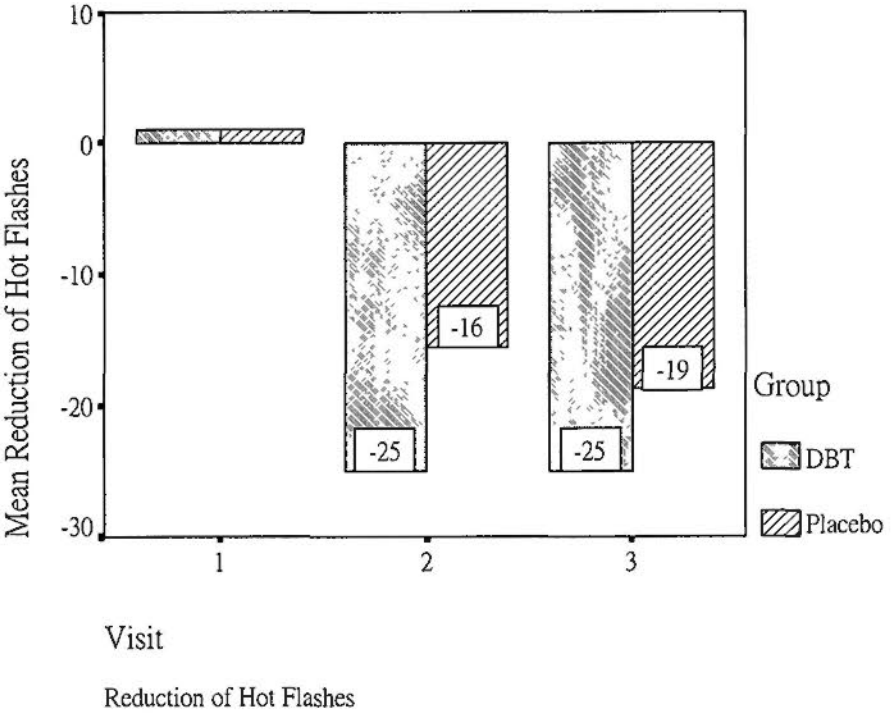

Figure 10 Reductions of Hot Flashes

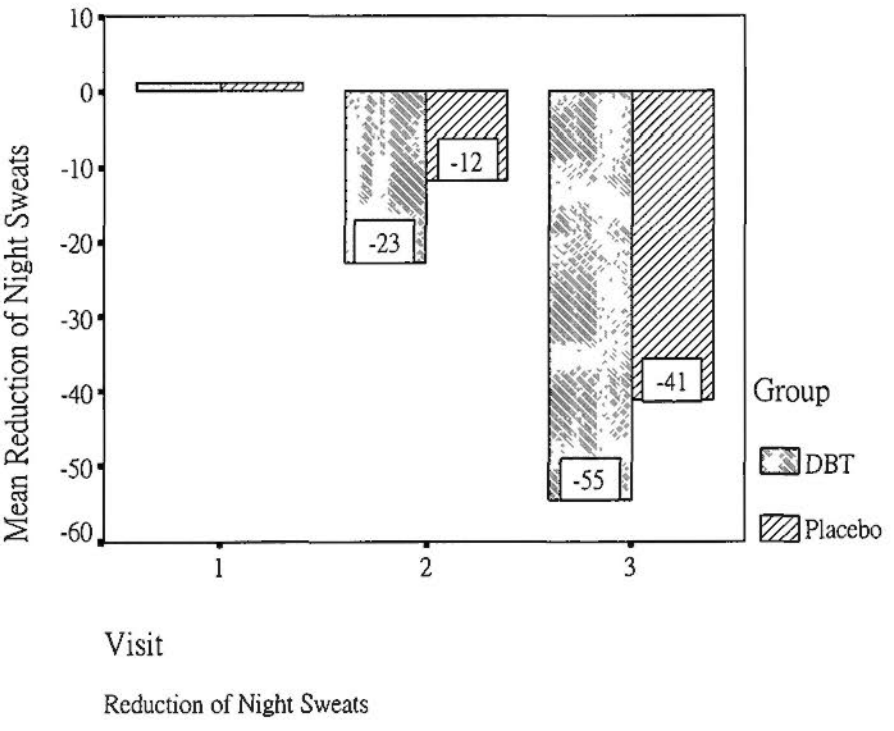

Figure 11 Reduction of Night Sweats

The incidence of subjects with vasomotor symptoms in DBT group was decreased when compared with placebo group during study period but without significance (p=0.098). However, the mean score of vasomotor domain in placebo group were

lower than DBT group (p=0.006).

For sexual domain, the mean scores in DBT group were significantly decreased at the end of study when compared with baseline (p=0.006), however there was no significant difference in placebo group between baseline and the end of study (p=0.069).

In DBT group, incidence of hot flash was decreased when compared with baseline (p=0.082), and incidence of Night Sweats was significantly decreased compared with baseline (p=0.008). No such changes were observed in Placebo group (Table 10, Figure 10, 11).

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4 Vaginal maturation

Table 6 Vaginal Maturation Index*

Parameters	Age group	Baseline	Visit 3	p-value (Baseline vs. visit 3)
DBT	< 50 years	36.42±21.29	26.50±12.62	0.894
	> 51 years	42.12±19.15	30.04±12.33	0.292
	All ages	40.45±19.70	28.98±12.19	0.411
Placebo	< 50 years	34.88±16.94	30.35±17.97	0.110
	> 51 years	31.45±19.68	25.11±22.91	0.073
	All ages	32.73±18.53	27.29±20.74	0.581
P value (DBT vs. Placebo)		0.074	0.774	

*Vaginal Maturation Index = (% Intermediate Cells × 0.5) + % Superficial Cells

For Vaginal maturation index (VMI), no differences between the two groups were observed during the study period.

5 Hormone levels

Table 7 Hormone Levels

Parameters	Study visit	DBT	Placebo	p-value
FSH				
All ages	Baseline	70.57±24.79	87.93±29.63	0.004
<50 years	Baseline	79.81±29.43	95.61±33.07	
>51 years	Baseline	66.39±21.64	82.41±27.24	
All ages	Visit 2	69.65±25.05	86.00±30.01	0.008
<50 years	Visit 2	78.15±30.49	96.20±33.57	
>51 years	Visit 2	65.81±21.64	79.35±26.08	
All ages	Visit 3	68.61±24.45	84.84±32.69	0.012
<50 years	Visit 3	79.39±29.77	98.12±34.30	
>51 years	Visit 3	63.75±20.36	76.18±29.14	
P value (visit 1 vs. visit 3)		< 50 years: 0.857 > 51 years: 0.098 all ages: 0.708	< 50 years: 0.334 > 51 years: 0.025 all ages: 0.700	
LH				
All ages	Baseline	33.91±12.20	42.54±14.97	0.005
<50 years	Baseline	39.77±16.05	46.73±14.29	
>51 years	Baseline	31.26±9.14	39.82±15.41	
All ages	Visit 2	31.61±12.42	39.65±14.40	0.008
<50 years	Visit 2	36.83±15.13	44.22±14.19	
>51 years	Visit 2	29.26±10.42	36.67±14.04	
All ages	Visit 3	32.43±12.27	39.44±15.11	0.022
<50 years	Visit 3	38.79±15.86	45.10±13.31	
>51 years	Visit 3	29.56±9.18	35.75±15.34	
P value (visit 1 vs. visit 3)		< 50 years: 0.567 > 51 years: 0.104 all ages: 0.093	< 50 years: 0.115 > 51 years: 0.005 all ages: 0.001	
E2				
All ages	Baseline	51.96±17.73	48.21±6.42	0.215
<50 years	Baseline	49.00±6.83	46.93±4.10	

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>51 years	Baseline	53.29±20.86	49.17±7.62	
All ages	Visit 2	47.42±5.43	47.50±6.46	0.953
<50 years	Visit 2	48.43±5.84	46.87±5.66	
>51 years	Visit 2	46.97±5.28	47.91±7.03	
All ages	Visit 3	47.76±6.48	48.11±5.22	0.790
<50 years	Visit 3	50.14±8.75	46.80±4.87	
>51 years	Visit 3	46.68±4.96	49.05±5.37	
P value (visit 1 vs. visit 3)		< 50 years: 0.666 > 51 years: 0.071 all ages: 0.113	< 50 years: 0.927 > 51 years: 0.885 all ages: 0.861	

The hormone levels (FSH and LH) were higher in placebo group than DBT group from baseline to the end of the study ($p<0.022$) (Table 7, Figure 6, 7). However, LH level in placebo group was significantly decreased at the end of study when compared with baseline ($p=0.001$) (Figure 9).

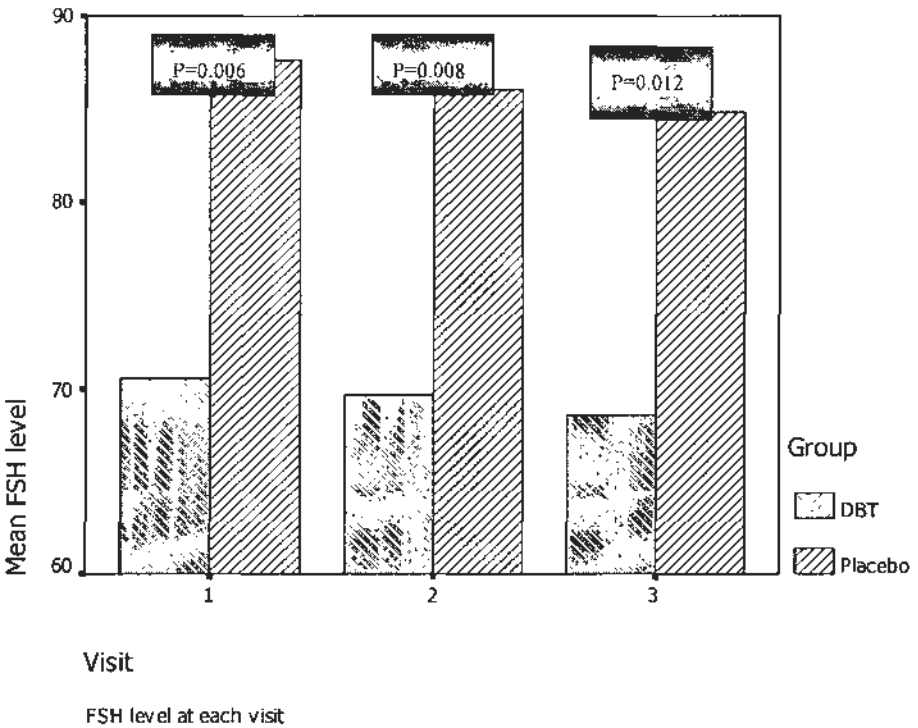

Figure 6 FSH Level Comparisons

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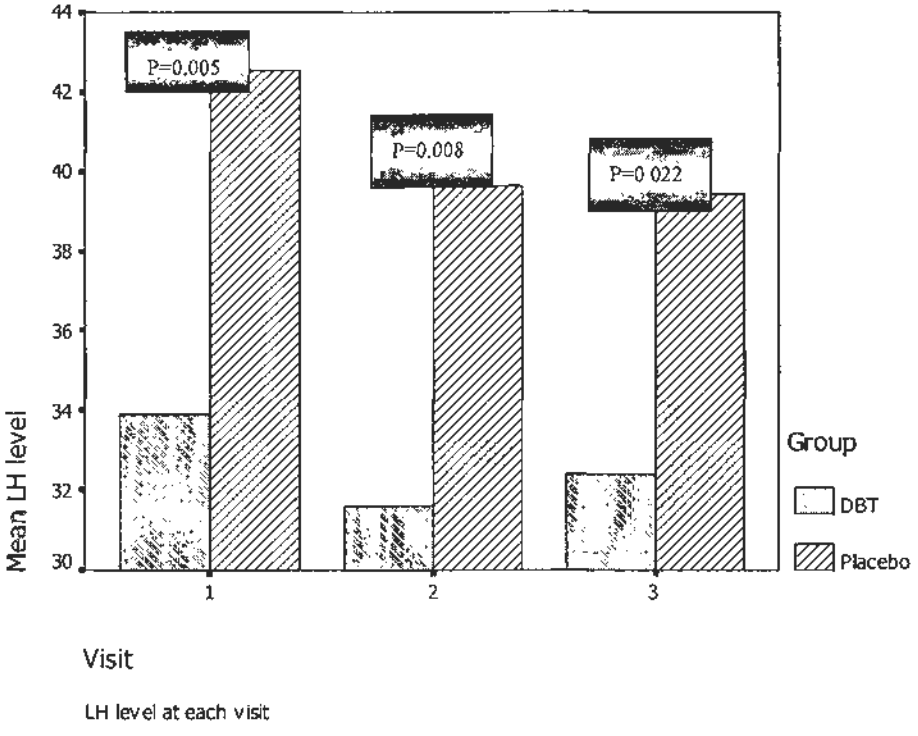

Figure 7 LH Level Comparisons

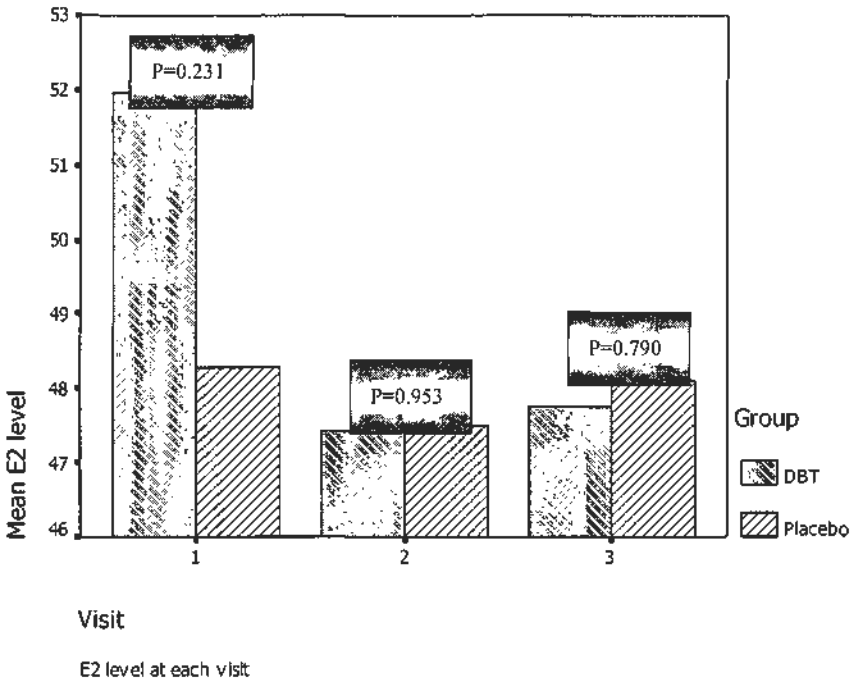

Figure 8 E2 Level Comparisons

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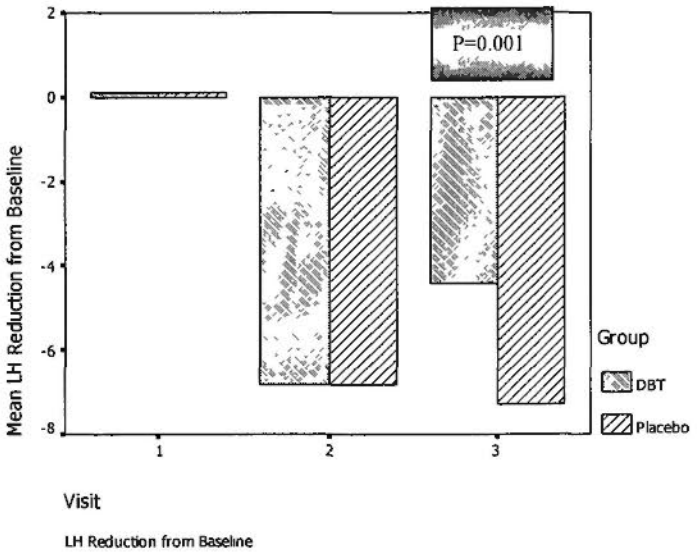

Figure 9 LH Reductions from Baseline

6 Blood chemistry

Table 8 Blood Chemistry

Test item	DBT (N=45)	Placebo (N=38)	P value
Alkaline Phosphates			
Visit 1	68.91±17.56	62.76±10.54	0.053
Visit 2	74.24±18.61	68.32±13.68	0.099
Visit 3	78.38±20.95	65.56±22.21	0.008
P value (visit 1 vs. visit 3)	0.000	0.413	
ALT/GPT			
Visit 1	24.24±12.92	25.61±15.59	0.665
Visit 2	22.58±8.90	23.34±10.26	0.717
Visit 3	22.62±10.60	24.71±12.17	0.406
P value (visit 1 vs. visit 3)	0.250	0.750	
Bilirubin			
Visit 1	10.69±4.20	9.89±3.13	0.339
Visit 2	11.51±4.65	9.87±2.75	0.050
Visit 3	11.64±4.91	10.37±3.51	0.185
P value (visit 1 vs. visit 3)	0.105	0.344	
Creatinine			
Visit 1	68.20±17.06	72.74±10.37	0.156
Visit 2	66.98±10.03	67.39±8.47	0.840
Visit 3	65.31±8.87	68.95±8.76	0.065
P value (visit 1 vs. visit 3)	0.196	0.004	
Cholesterol			
Visit 1	5.52±0.90	5.53±0.94	0.966
Visit 2	5.49±0.84	6.46±0.84	0.896
Visit 3	5.47±0.86	6.88±7.91	0.238
P value (visit 1 vs. visit 3)	0.605	0.292	

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7 Withdrawn case

Table 11 Withdrawn cases

Reason of withdrawn	DBT	Placebo
Headache & hotness		1
Chest pain & constipation	1	
PR bleeding (SAE)	1	
Insomnia, headache & stomach upset		2
Epigastric discomfort	1	2
Chest discomfort & palpitation		1
Prolong flu		1
Skin tags on neck and upper chest		1
High cholesterol	1	
Asymptomatic increase in hepatic enzyme		1
Not help		1
Violation of protocol	1	
Pituitary edenoma		1
Total	**5**	**11**

8 Adverse Events

Table 9 Adverse Events

Adverse Event	No. (%) of Participants							
	DBT (n= 45)				Placebo (n= 38)			
	Visit 1	Visit 2	Visit 3	Total	Visit 1	Visit 2	Visit 3	Total
Urinary frequency	1			1				
Dysuria		1		1				
Stomach discomfort	1			1	1			1
Epigastric discomfort	2			2	1			1
Itch skin	1			1				
Haematura	1			1				
Breast lump	1			1				
Dizziness	1			1				
Hot feeling in throat	1			1	1			1
Sore throat					1		1	2
Hot flushes					1			1
Headache					1			1
Borderline hypertension					1			1
Tinnitus					1			1
Worsening of pre-existent bilateral wrist elbow pain					1			1
Dry mouth		1		1	1			1
Palsitations					1			1
Vaginal discharge						1		1
Numbness left hand						1		1
Total	9 (20%)	2(4.4%)	0	**11(24%)**	11(28.9%)	2(5.3%)	1(2.6%)	**14(37%)**

The overall adverse events (24%) in DBT group were lower than placebo group (37%) (Table 9).

9 Conclusions

1) Mild hot flashes were significantly decreased after DBT treatment, no significant reduction in placebo group was observed.
2) The incidence of subjects with vasomotor symptoms was decreased in DBT group during study period.
3) Mean score of Vasomotor Domain in placebo group was decreased, but no changes were observed in DBT group.
4) Mean score of sexual domain in DBT group were significantly decreased, no changes were seen in placebo group.
5) Hormone (FSH and LH) concentrations in placebo group were higher than DBT group from baseline to the end of study.
6) In placebo group, LH level of age > 51 years and LH level were significantly decreased compared with baseline, however, no such changes were observed in DBT group.
7) The adverse events in DBT were lower than placebo (24% vs. 37%)

10 Suggestions

According to the data of the study, if further clinical trial is considered, a stratified randomization is recommended to control the factors such as hormone levels, age, duration of menopause, education level and economic status etc. these factor may influence the results of the clinical trial.